

MINUTES OF THE 46th GENERAL ASSEMBLY OF THE EUROPEAN ASSOCIATION FOR THE STUDY OF DIABETES

held in Stockholmsmässan, Stockholm, Sweden on 23 September 2010

Present:	Dr. U. Smith	(President)
	Dr. G. Spinas	(Honorary Treasurer)
	Dr. M. Stumvoll	(Honorary Secretary)
	Dr. E. Gale	(Editor-in-Chief, Diabetologia)
	Dr. J. Nolan	(Chair, PGEC)
	Dr. V. Jörgens	(Executive Director)
	Dr. M. Grüsser	(Vice Director)
	and 51 members	

The Vice President, Dr. Boulton, welcomed everyone to the 46th General Assembly as the President had unexpectedly to attend a press conference.

was held on 6 September 2010 with representatives from ESC, ERS, ECCO and EASD. Dr. Smith thanked all members of the Programme Committee 2010 for their commitment and hard work.

1. MINUTES 45th GENERAL ASSEMBLY 2009

Since there were no comments, the minutes were approved unanimously and officially signed as a correct record.

2. REPORTS

a) President

The President's report to the members on the activities of EASD was given in the President's Address before the Minkowski Lecture. It is available under:

<http://easd.conference2web.com/content/590>

This report covered the following topics:

The President reported on the various activities and grant programmes of the Foundation and expressed his thanks to all partners. He reported that the DIAMAP project had been finalised and the results had been presented to the international press during the Annual Meeting. He said the postgraduate courses continued to be successful and attracted many young researchers. Dr. Smith reported that recently the Alliance for Biomedical Research in Europe had been founded 'to promote the best interests and values of researchers across all medical disciplines in Europe, in those general areas where common interest is identified' (quoted from the Mission Statement). He reported that the founding assembly of the Alliance

b) Honorary Treasurer

Dr. Spinas reported that the income had increased slightly in 2009 due to the payment from Interplan for the Rome Annual Meeting. He said expenditure had decreased in 2009 because no transfer of funds had been made to the Foundation. This was done in the early part of 2010.

Dr. Spinas expressed his thanks to Drs. Grüsser and Jörgens for their support and advice and to the team in Düsseldorf in general and to Mrs. Klee, Ms. Deparade and Ms. Weiss in particular for the precise handling of the accounts.

(The President took over the General Assembly)

The President thanked the Honorary Treasurer for his diligence and asked if there were any questions. Dr. A. Pfeiffer queried if there were too many assets. Dr. Spinas explained that these assets were planned for and were all allocated. There were no further comments.

c) Honorary Auditors

The President asked the Honorary Auditors, Drs. Pater-son and Tack, for their report. Dr. Tack confirmed that the accounts had been checked carefully and were in

perfect order. Dr. Smith asked for the vote to accept the accounts.

The Honorary Treasurer was unanimously discharged (42 votes for and 5 abstentions).

d) Honorary Secretary

Dr. Stumvoll reported that 2159 abstracts had been submitted; of the 1352 which were accepted 264 were orals. The top three countries for abstract submission were USA, UK and Italy and the top three countries for abstract acceptance were USA, UK and Sweden.

Dr. Stumvoll closed his report by thanking all members of the EASD staff, in particular Ms. H. Goliberzuch and Mrs. M. Toledo, for their outstanding help and support with the organisation of the EASD Annual Meetings.

Dr. Smith thanked Dr. Stumvoll for his diligence and asked if there were any questions. Dr. Halban expressed his sincere thanks to Dr. Stumvoll for a fantastic job done. No further questions were asked.

e) Editor-in-Chief, Diabetologia

Dr. Gale briefly reported that the impact factor of Diabetologia was steadily increasing and the journal continued to be successful. As his term of office was coming to an end, he thanked the Executive Committee for their support and said it had been a tremendous privilege for him to serve as Editor-in-Chief for 7 years. He also expressed his thanks to the team in Bristol and handed over to Dr. Zierath and Dr. Nolan and wished them both all the best.

Dr. Smith thanked Dr. Gale for his dedication, active involvement in and contribution to the journal.

f) Chair, Postgraduate Education Committee

Dr. Nolan reported that a second course had been held in Kiev in April 2010 and another course was being planned in Ukraine in April 2011. The 3rd Oxford - Thessaloniki Diabetes Forum for 2011 would also be endorsed as an 'under the auspices of EASD' course. Another successful Minkowski EASD Advanced Postgraduate Course in Clinical Diabetes had been held in Wroclaw, Poland in March 2010. Future courses were being planned in Belgrade (Serbia), Riga (Latvia) and Sarajevo (Bosnia & Herzegovina).

Dr. Nolan reported that the web education sessions were progressing very well. Dr. Nolan will continue to plan

these activities until Dr. Kerr returns from his sabbatical.

Dr. Nolan closed his report by thanking the EASD staff for their friendly assistance. Dr. Smith thanked Dr. Nolan for his diligence in his role as Chair of the Postgraduate Education Committee.

g) Chair, Extra-European Postgraduate Activities

Dr. Boulton reported on the successful ventures in India: The Best of EASD India Diabetic Foot Seminars and the annual Best of EASD-India Courses. The 5th EASD/ADA/IDF Advanced Postgraduate Course held in Dar es Salaam, Tanzania had been very well attended. Dr. Boulton reported that courses were being planned in Nepal in November 2010 and in China in December 2010. Another Best of EASD-India Course will be held in Cochin and Bangalore in February 2011. Dr. Boulton said he was looking into the possibilities of holding courses in Addis Ababa and Cuba in 2011.

Dr. Smith thanked Dr. Boulton for his hard work in organising the extra-European activities.

3. ELECTIONS

Extension, Honorary Treasurer (2010 - 2011)

The General Assembly unanimously approved Dr. Spinass' extension until 2011, with 42 votes and 1 abstention.

Honorary Secretary (2010 - 2013)

The election of Dr. M. Walker was unanimously approved with 44 votes and 1 abstention.

Chair, PGEC (2010 - 2013)

The election of Dr. C. Tack was unanimously approved with 50 votes and 1 abstention.

Council Members (2011 - 2014)

The election of Drs. K. Birkeland, H. J. Bodansky, C. Levy-Marchal and P. Nawroth was unanimously approved with 49 votes and 1 abstention.

Honorary Auditor (2010 - 2013)

The election of Dr. M. Roden was unanimously approved with 49 votes and 1 abstention.

4. STUDY GROUPS

Dr. Boulton reported that he, together with Dr. Bosch, had taken over the organisation of the EASD Study Groups and representatives from all of them had been invited to a forum during the Annual Meeting. At the forum he had suggested that the Study Groups supply him with a list of their most experienced speakers which he could make use of for the postgraduate courses. He also proposed that the Study Groups make suggestions to the Honorary Secretary for symposia during the Annual Meeting; he had of course emphasised that the decision would lie with the Honorary Secretary regarding the final programme. At the Forum he had reiterated that the Study Groups did not receive financial support from the EASD.

5. HONORARY MEMBERSHIP

The nominations for Drs. C. Mogensen and P. Zimmet were unanimously approved.

6. ANY OTHER BUSINESS

Dr. Smith thanked the industry for their support. He also expressed his sincere gratitude to the Local Organising Committee for their outstanding contribution to the organisation of the 46th EASD Annual Meeting. He again thanked Drs. Stumvoll, Gale and Nolan for their hard work. The President warmly thanked Dr. Jörgens and the EASD team in Düsseldorf for their dedicated work.

Dr. Smith brought the General Assembly to a close at 18:55.

Agenda for the 47th General Assembly of the European Association for the Study of Diabetes

to be held in the Sanches Hall, Lisbon International Fair, Portugal on Thursday 16 September 2011 at 18:00

1. Minutes of the 46th General Assembly, Stockholm, Sweden 2010

2. Reports

a) President	U. Smith
b) Honorary Treasurer	G. Spinass
c) Honorary Auditors	M. Roden
	K. Paterson
d) Honorary Secretary	M. Walker
e) Editor-in-Chief, Diabetologia	J. Zierath
f) Chair, Postgraduate Education Committee	C. Tack
g) Chair, Extra-European Postgraduate Activities	A. J. M. Boulton

3. Elections

a) President (2011-2014)	in place of U. Smith
b) Vice President (2011 – 2014)	in place of A. J. M. Boulton
c) Honorary Treasurer (2011 – 2014)	in place of G. Spinass
d) Council Members (2012 - 2015)	in place of J.-M. Boavida C.-G. Östenson A. Pfeiffer N. Wareham
e) Honorary Auditor (2011 - 2014)	in place of K. Paterson in place of M. Roden

4. Study Groups

F. Bosch
A. J. M. Boulton

5. Honorary Membership

6. Any other business

47th EASD Annual Meeting of the European Association for the Study of Diabetes

Lisbon, Portugal, 12–16 September 2011

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- OP 08 Stem cells and regeneration
- OP 09 Prediction of complications in type 2 diabetes
- OP 10 Exploring mechanisms of insulin resistance
- OP 11 Education
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- PS 007 Epidemiology and prediction of type 2 diabetes
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OP 01 Assessing cardiovascular risk

1

An examination of multiple cardiovascular endpoints in individuals with screen-detected type 2 diabetes in the ADDITION-Europe trial: intervention effects and methodological insights

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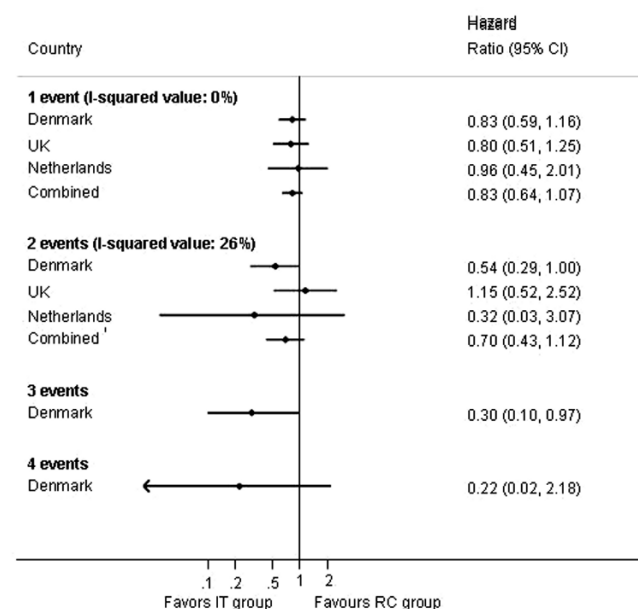
¹MRC Epidemiology Unit, Cambridge, UK, ²School of Public Health, Aarhus, Denmark, ³Institute of Public Health, Odense, Denmark, ⁴Department of Cardiovascular Sciences, Leicester, UK, ⁵Department of Health Sciences, Leicester, UK, ⁶Julius Center for Health Sciences and Primary Care, Utrecht, Netherlands.

Background and aims: Intensive treatment of multiple cardiovascular (CV) risk factors can halve mortality among people with established type 2 diabetes. The modest but significant increases in the intensity of treatment in the *ADDITION-Europe* trial were not associated with a reduction in first CV events in screen-detected individuals during five years of follow-up. We aimed to (i) describe the total CV burden in *ADDITION-Europe*; (ii) quantify the impact of the intervention on multiple CV events; and (iii) explore the methodological implications of using first or multiple events to estimate effects.

Materials and methods: In a pragmatic, cluster-randomised, parallel group trial in four centres (Denmark, Cambridge UK, the Netherlands and Leicester UK), 343 general practices were randomised to screening plus routine care of diabetes according to national guidelines (RC; 1,379 patients), or screening and promotion of target-driven, intensive treatment of multiple risk factors (IT; 1,678 patients). The Wei, Lin, and Weissfeld method was used to estimate the effect of the intervention on the hazard of independently adjudicated cardiovascular events (cardiovascular mortality, cardiovascular morbidity, revascularisation, and non-traumatic amputation) over a mean follow-up of 5.3 years.

Results: 238 individuals had a first CV event, 71 a second event, and 25 three or more events. The most frequent first events were revascularisations (n=88; 37%), non-fatal MI (n=61; 26%) and CV death (n=48; 20%). The corresponding numbers for second events were 55 (77%), 5 (7%) and 8 (11%) respectively. There was a 17% reduction in the risk of a first primary endpoint (HR 0.83, 95% CI: 0.65 to 1.05). Reduction in the risk of a second primary endpoint was 30% (HR 0.70, 95% CI 0.43 to 1.12).

Conclusion: An intervention achieving modest changes in treatment of screen-detected patients was not associated with significant reductions in the incidence of first or second CV events over five years. Focussing on first events in CVD prevention trials underestimates the total CV burden to patients and the health service. However, the lack of independence between many of the first and second CV events suggests that we should continue to focus on first events when estimating effects of interventions on composite endpoints.



Clinical Trial Registration Number: NCT00237549

Figure 1 The effect of the intervention on the hazard of experiencing 1, 2, 3 and 4 events. Each intervention effect is presented as a hazard ratio within each country and combined across countries using fixed-effects meta-analysis.

2

Lower incidence of cardiovascular events in a recently recruited cohort of Japanese type 2 diabetes patients in primary care settings

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¹Oishi clinic, Kyoto, ²Jiyugaoka Medical Clinic, Obihiro, ³Kawai Clinic, Tsukuba, ⁴HEC Science Clinic, Yokohama, ⁵Takeda Clinic, Isehara, ⁶Minami Masae Clinic, Fukuoka, ⁷Sugimoto Clinic, Kitakyushu, ⁸University of Tsukuba, ⁹Division of Clinical Epidemiology, Jikei University School of Medicine, Tokyo, ¹⁰Shiga University of Medical Science, Otsu, Japan.

Background and aims: Major changes in treatment regimens over the past years, with more stringent goals for metabolic control and cardiovascular risk management, may yield improvement of cardiovascular morbidity in subjects with type 2 diabetes. The aim was to investigate whether a reduced incidence of cardiovascular disease can be achieved in a newly recruited cohort following the recently advanced concept of multifactorial treatment and followed in primary care settings as compared with previously reported cohorts.

Materials and methods: A prospective study was performed at 17 nationwide clinics of the Japan Diabetes Clinical Data Management (JDDM) study group. Subjects were 2,984 patients with type 2 diabetes without prevalent cardiovascular disease. The main outcome was non-fatal or fatal coronary heart disease, ischemic stroke, or peripheral artery disease and the incidence was compared with previously reported cohorts. The following 4 cohorts dealing with type 2 diabetes without prevalent cardiovascular disease with an observation period of more than 4 years were employed to compare: the Diabetes and Informatics Study Group (DAI), Atherosclerosis Risk in Communities Study (ARIC), Fenofibrate Intervention and Event Lowering in Diabetes Study (FIELD) and United Kingdom Prospective Diabetes Study (UKPDS).

Results: There were 90 cardiovascular events over 10,827 person years of follow-up with a drop-out rate of 6%. The incidences (per 1,000 person years) of composite, coronary heart disease, ischemic stroke and peripheral artery disease in the JDDM were 8.3, 4.4, 3.1, and 0.7, respectively. As shown in the table, each incidence was lowest in the JDDM compared with other cohorts ($p < 0.01$ vs. each cohort). In the JDDM, significant variables predictive of the occurrence of a cardiovascular event were age, duration of diabetes, hemoglobin A_{1c}, high density lipoprotein cholesterol, and urinary albumin by Cox multivariate regression analysis.

Conclusion: The incidence of cardiovascular disease is likely to be reduced in patients with type 2 diabetes followed in primary care settings with a recently-advanced concept of treatment as compared to earlier large-scale cohorts. The novel finding of low cardiovascular disease occurrence may be conferred by the feasibility at primary care settings for providing patients with type 2 diabetes with favorable controls in blood glucose, blood pressure, and lipids, coupled with unique ethnicity/country factors.

Cardiovascular outcomes in different cohort studies of patients with type2 diabetes

	JDDM	DAI	ARIC	FIELD	UKPDS
Number of patients	2,984	11,644	1,460	9,795	2,693
Recruitment year	2004	1998	1987	1998	1977
Incidence(per 1,000 person-years, 95% CI)					
Coronary heart disease	4.4	30.3	10.8	11.1	13.2
Stroke	3.1	NA	5.6	6.8	6.1
Peripheral artery disease	0.7	NA	NA	2.3	4.2

Supported by: JDS

3

Comparison of ACCORD trial outcomes with outcomes estimated from modelled and meta-analysis studies

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¹IMS Health, Basel, Switzerland, ²IMS Health, London, ³University College London, UK, ⁴University of Michigan, Ann Arbor, USA.

Background and aims: The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial reported significantly higher mortality in type 2 diabetes patients treated with intensive glucose lowering (target HbA1c < 6%) versus standard treatment (target HbA1c 7% - 7.9%). These findings have been interpreted as changing our understanding of the relationship between HbA1c and Cardiovascular (CV) risk at levels of HbA1c below 7. We wished to put the ACCORD findings in context by comparing them with outcomes estimated from other data sources.

Materials and methods: The IMS CORE Diabetes model (CDM) is a validated and widely used computer simulation model that predicts the development and consequences of diabetes complications. The CDM uses data from the UKPDS to predict the relationship between HbA1c and cardiovascular end points as well as all cause mortality. UKPDS risk equations assume that continuing to reduce HbA1c below 7 brings continued reduction in CV risk. We compared outcomes observed in the ACCORD trial with i) predictions from the CDM when the baseline characteristics of the ACCORD population were entered into the model and UKPDS equations were used; ii) predictions by the CDM as above but after modifying the UKPDS equations so that continuing to reduce HbA1c below 7 causes no further reduction in CV risk; iii) a random effects meta analysis of data from three recent large trials: ACCORD, ADVANCE and VADT.

Results: Relative risks of non fatal MI; non fatal stroke; heart failure and all cause mortality are shown in the table. Relative risk of all cause mortality in the intensive treatment group of ACCORD was significantly higher than that predicted by the model based on UKPDS. However, for non fatal stroke, non fatal AMI, and heart failure the model predictions were within 95% CIs derived from ACCORD. Adjusting the model so continuing to reduce HbA1c below 7 causes no further reduction in CV risk brought the model estimates closer to the ACCORD findings for all outcomes, but the difference in all cause mortality remained outside the confidence interval. Predictions from the adjusted model fell within confidence intervals predicted by the meta-analysis for all four events. Table: Relative Risk of important DM events from different data sources (intensive vs standard treatment, point estimate, 95% CI)

Outcome	ACCORD observed	Modelled CDM (i)*	Modelled CDM (ii)**	ACCORD/ADVANCE/VADT Pooled
Non fatal MI	0.79 (0.62-0.92)	0.82	0.76	0.86 (0.74-0.99)
Non fatal stroke	1.10 (0.75-1.5)	0.91	0.83	0.97 (0.83-1.12)
Heart failure	1.23 (0.93-1.49)	0.97	0.93	1.03 (0.86-1.23)
All cause mortality	1.27 (1.01-1.46)	0.95	0.94	1.07 (0.87-1.32)

* HbA1c benefits from UKPDS: ** Continuing to reduce HbA1c below 7 causes no further reduction in CV risk

Conclusion: Relative risk of all cause mortality in ACCORD was significantly different from that modelled based on previous data. This difference was not seen in other endpoints, was not consistent across recent studies, and was not fully explained by hypothesising a lower limit of 7 for HbA1c benefit. Observed mortality effects in the ACCORD trial may be related to factors other than glucose lowering.

4

An improved model to estimate lifetime health outcomes of patients with type 2 diabetes using 30-year follow-up data from the United Kingdom prospective diabetes study

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¹Sydney School of Public Health, University of Sydney, Australia, ²Health Economics Research Centre, University of Oxford, ³Diabetes Trials Unit, University of Oxford, UK.

Background and aims: To extend and enhance the existing United Kingdom prospective diabetes study (UKPDS) Outcomes simulation model for type 2 diabetes by using additional patient-level data from the ten-year post-trial monitoring phase of the UKPDS to re-estimate risk equations for diabetic complications and death.

Materials and methods: Equations for forecasting the occurrence of ten diabetes-related complications and death were estimated using data from 5102 UKPDS patients followed for up to 30 years. Covariates included in the risk equations were demographic characteristics, smoking status, classical risk factors (systolic blood pressure, HbA1c, lipid levels and history of other comorbidities). New risk factors examined as potential covariates were micro/macro albuminuria, heart rate, white blood cell count, haemoglobin and estimated glomerular filtration rate. Also available as updated, rather than just baseline covariates, were peripheral venous disease (PVD), body mass index and atrial fibrillation. In addition to the six complications modelled previously (1st myocardial infarction (MI), 1st stroke, ischaemic heart disease, heart failure, blindness and renal failure) and death, we have now included diabetic ulcer, renal impairment, 2nd MI and 2nd stroke. Parametric proportional hazards models equations were estimated to predict absolute and relative risks associated with each event type. These were then combined in a simulation model to predict event rates, life expectancy and quality adjusted life expectancy for individuals and populations with different characteristics.

Results: All event equations utilised more than 60,000 patient years of data. For each of the original outcomes we have approximately twice as many total events as used in the estimation of the previous risk equations, for example the 1st MI equation has 925 events compared with 495 previously. The additional outcomes are 175 2nd MIs, 47 2nd strokes, 86 ulcer events and over 2000 cases of renal impairment. In terms of the new risk factors, micro/macro albuminuria was a significant covariate in 8 of the equations with hazard ratios (HR) ranging from 1.3 (95%CI 1.1-1.5) for death to 4.8 (95%CI 2.8-8.5) for renal failure. PVD was a significant covariate in 7 equations with HRs ranging from 1.3 (95%CI 1.1-1.5) for death to 6.3 (95%CI 2.9-6.9) for amputation. White blood cell count was a significant covariate in 4 equations with HRs ranging from 1.05 (95%CI 1.02-1.07) for 1st stroke to 1.06 (95%CI 1.03-1.10) for blindness. Notably, atrial fibrillation was associated with a high risk of 1st stroke (HR 4.4, 95%CI 2.9- 6.6) and heart failure (HR 4.7, 95%CI 2.7-8.2).

Conclusion: The new UKPDS Outcomes Model, with its additional risk factors and increased number of events, can simulate a wider range of long-term outcomes and more reliably estimate risk, particularly for older people with diabetes. Improved prediction of likely clinical outcomes will permit more accurate future economic evaluations of interventions in type 2 diabetes.

Clinical Trial Registration Number: ISRCTN 75451837

Supported by: UK MRC; Australian NHMRC grants 512463, 571372 and 571122

5

Calculating risk in patients with type 2 diabetes in The Netherlands: UKPDS versus ZODIAC risk engine performance (ZODIAC-26)

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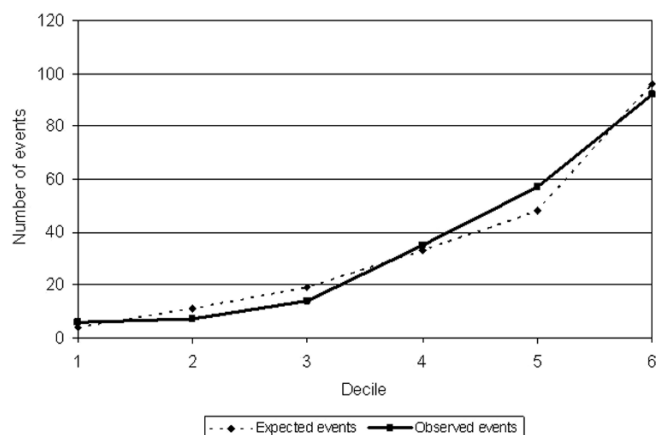
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Background and aims: Risk prediction models obtained in samples from the general population do not perform well in type 2 diabetic patients. Therefore, specific diabetes risk engines such as the UKPDS risk engine have been developed. This study presents an alternative diabetes-specific equation for estimation of the absolute 10 year risk of fatal cardiovascular disease (CVD) in type 2 diabetic patients and compares it to the more commonly used UKPDS. **Materials and methods:** This study was based on the ZODIAC cohort, including 1353 diabetes patients treated in primary care in the Netherlands,

with a median follow-up period of 9.6 years. The UKPDS risk engine was evaluated by comparing observed CVD mortality to expected CVD mortality as calculated by the UKPDS risk engine. The alternative risk equation was calculated with Cox proportional hazard models. Calibration was tested with the Hosmer-Lemeshow test, and discrimination capacities were calculated with the C-statistic. Possible predictors were: age, gender, smoking, blood pressure, BMI, diabetes duration, HbA1c, creatinine, albumin to creatinine ratio, macrovascular complications, lipid lowering therapy and antihypertensive medication use.

Results: During a median follow-up of 10 years, 570 (42%) patients died, 280 of whom died of cardiovascular disease (49%), average age at baseline was 68 (SD 12) years, and average HbA1c was 7.5% (SD 1.2). The predicted UKPDS fatal event rate was 29% as opposed to the observed rate of 18%. The alternative risk calculator eventually incorporated age, HbA1c, smoking, serum creatinine, urinary albumin to creatinine ratio and macrovascular complications. Calibration was sufficient, May and Hosmer test of fit; p-value = 0.29 (see figure 1) and discrimination was excellent with a C-statistic of 0.80. 10-year risk (CVD) = $1 - \exp\left\{-(0.089 \cdot \text{age}-60) + (0.418 \cdot \text{smoking}) + (0.230 \cdot \text{HbA1c}-7) + (0.647 \cdot \text{macrovascular complications}) + (0.016 \cdot \text{creatinine}-80) + (0.233 \cdot \log \text{urinary albumin creatinine ratio})\right\}$

Conclusion: The UKPDS risk engine overestimated CVD mortality risk considerably in this population. An alternative risk equation, which includes creatinine and albuminuria and excludes lipid profile and blood pressure, is nowadays probably more precise in type 2 diabetes patients treated in primary care in The Netherlands. Our model needs external validation. Figure. Observed and Expected deaths from cardiovascular disease.



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Health-related quality of life predicts survival in patients with type 2 diabetes and myocardial infarction: a report from the DIGAMI2 trial

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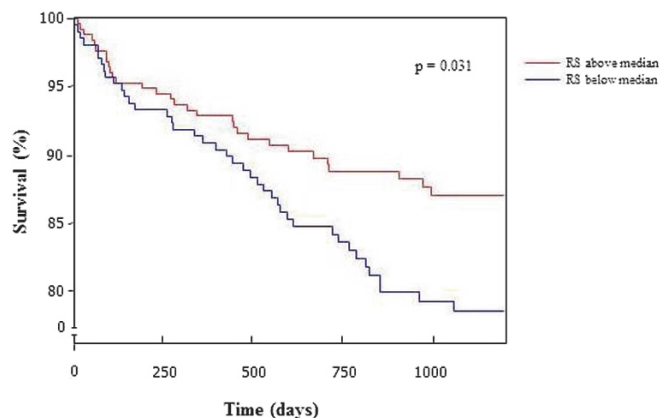
Background and aims: Diabetes is a major risk factor for cardiovascular (CV) disease and has a considerable impact on survival as well as health-related quality of life (HRQL). A prognostic link between self-reported HRQL and unfavourable outcome has been reported but is not well explored in patients with type 2 diabetes (T2DM) and acute myocardial infarction (AMI).

Materials and methods: The DIGAMI-2 HRQL sub-study recruited 509 AMI patients with T2DM (median age 67.7 years, interquartile range 59.9–74.1; male 68%). Self-reported HRQL was evaluated at hospital admission by a graded rating scale (RS) where 0 is death and 100 is perfect health. The median follow-up was 2.1 years (interquartile range 1.03–3.00). Prospective associations between all-cause, CV death (CVD) and CV events (CVE = CVD, nonfatal AMI or stroke) were assessed applying logistic regression.

Results: During follow-up 152 (30%) patients had a CVE and 86 (17%) died (70 CVD and 16 other). The RS scores at admission were significantly lower in patients who experienced a CVE (69 ± 19 vs. 62 ± 18 , $p < 0.001$), CVD (68 ± 19 vs. 62 ± 18 , $p = 0.012$) or all-cause mortality (68 ± 18 vs. 62 ± 19 , $p < 0.01$) than in those free from events. The RS score was a significant predictor for CVE (Odds ratio (OR) per 10 units increase in RS: 0.83, 95% Confidence Interval (95%CI): 0.75–0.93), CVD (OR: 0.85, 95%CI: 0.73–0.98) and all-cause mortality (OR: 0.84, 95%CI: 0.74–0.96). Women scored somewhat lower than men (64 ± 20 vs. 68 ± 19 ; $p = 0.034$). The RS score significantly predicted CVE (OR:

0.81, 95%CI: 0.71–0.93), CVD (OR: 0.80, 95%CI: 0.68–0.95) and all-cause mortality (OR: 0.77, 95%CI: 0.66–0.91) in men, however, not in women.

Conclusion: A low RS score is of prognostic significance in patients with type 2 diabetes and acute myocardial infarction thereby serving as an easily obtained indicator of patients at high risk for all-cause and CV mortality. The predictive value seems to be higher in men.



Clinical Trial Registration Number: 96-164

OP 02 Diabetic foot and acute osteoarthropathy

7

Potential explanation for lower incidence of foot ulceration in Asian compared to European patients with type 2 diabetes

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Background and aims: Diabetic patients of South Asian origin in the UK have about one-third the risk of foot ulceration compared to Europeans. Neuropathy and vascular disease are primary determinants of the risk of foot ulceration. To define differences in predisposing risk factors for ulceration, 73 South Asian (age: 60 ± 11 ; duration of diabetes: 12.9 ± 0.769 ; BMI: 30.1 ± 0.6) and 79 European (age: 63 ± 8 , duration of diabetes: 11.9 ± 0.7 ; BMI: 33.6 ± 0.6) patients with Type 2 diabetes underwent assessment of neuropathy using: neuropathy symptoms (NSP) and severity (NDS), electrophysiology (NCS), Vibration Threshold (VPT), Thermal threshold (warm and cold sensation), heart rate variability (HRV), Neuropad, and corneal confocal microscopy. Vascular integrity was assessed using laser Doppler and TCPO2.

Results: Comparing Asians with Europeans: They had a significantly higher HbA1c (8.2 ± 0.2 v 7.6 ± 0.2 , $P=0.02$) but comparable BP: Systolic/Diastolic ($133/72 \pm 1.9$ v $135/70 \pm 1.9$), $P=0.56$). Triglycerides were significantly lower in Asians (1.9 ± 0.11) compared to Europeans (2.4 ± 0.2), $P=0.008$, whilst HDL and total cholesterol were comparable. NSP (7.0 ± 0.8 v 6.5 ± 0.6), NDS (3.4 ± 0.4 v 3.8 ± 0.3 , $P=0.43$) and VPT (14.7 ± 1.2 v 17.5 ± 1.3), $P=0.10$) were comparable. Sural conduction velocity (45.5 ± 0.9 v 44.9 ± 0.81) was comparable but amplitude (11.8 ± 1.0 v 8.3 ± 0.7 , $P=0.004$) was better in Asians. WS (41.6 ± 0.5 v 42.0 ± 0.4 , $P=0.54$), CS (24.4 ± 0.7 v 23.9 ± 0.6 , $P=0.62$) did not differ. Corneal confocal microscopy showed no difference in corneal nerve fibre density (23.7 ± 1.6 v 24.8 ± 1.1 , $P=0.56$ or tortuosity (17.5 ± 0.7 v 20.4 ± 0.9 , $P=0.39$) but nerve fibre length (19.5 ± 1.2 v 18.3 ± 0.8 , $P=0.03$ and nerve branch density (36.1 ± 4.1 v 28.1 ± 2.0 , $P=0.05$) were better in Asians. HRV response to deep breathing was non significantly better in Asians compared to Europeans (8.3 ± 0.7 v 6.9 ± 0.5 , $P=0.11$). TCPO2 was significantly higher in Asians (63.2 ± 1.3) v Europeans (57.6 ± 1.43), $P=0.005$. The laser Doppler hyperaemic response (648.1 ± 53.4 v 531.7 ± 34.9), $P=0.06$ was higher in Asian compared to European diabetic patients.

Conclusion: In conclusion we demonstrate that despite worse glycaemic control, Asian patients have better small fibre function and structure compared to European patients with Type 2 diabetes. Furthermore they have higher foot skin oxygenation and hyperaemic blood flow response to heating, which may protect from the development of foot ulceration.

Supported by: Diabetes UK

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Imaging by white light absorption spectroscopy to assess peripheral limb blood flow in diabetes

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Background and aims: When white light is reflected back from skin, its spectral characteristics depend on the oxygenation of the blood in the soft tissues, and this can be determined using hyperspectral (HS) imaging. The use of the technique has hitherto been hampered by the scattering of light which makes the relationship between absorption and attenuation non-linear. The aim of this study was to determine if a new algorithm might enable the use of HS imaging to predict clinical outcome in diabetic foot disease. **Materials and methods:** The method was validated *in vitro* by bubbling nitrogen through blood from healthy volunteers and measuring oxygen saturation both by blood gas analysis and by HS imaging of a sample trapped between two microscope slides. HS imaging was also undertaken in patients with diabetic foot ulcers presenting consecutively to a specialist out-patient

centre, and an association was sought clinical outcome at 12 and 24 weeks. The studies had ethical committee approval and participants gave written informed consent. Clinical researchers and those analysing the HS images were each blind to the results of the other.

Results: There was a very close positive correlation ($r=0.9939$, linear regression) between *in vitro* HS assessment of oxygenation in blood of known saturation between 2% and 100%. An index ulcer was selected in 43 people newly presenting to the clinic. Mean age was 62.7 (SD 12.2) years; 12 women, 31 men; 6 type 1 DM, 37 type 2. 18 ulcers were infected. There was no difference in oxygenation assessed by HS imaging at different sites on the foot at baseline, and no correlation between HS imaging and either ABPI or skin temperature. 26 ulcers healed by 12 weeks. There was a negative correlation between healing by this time and the results of baseline HS imaging ($r=-0.392$, $p=0.009$; Pearson): indicating that greater degrees of oxygenation of haemoglobin were associated with worse clinical outcome. Regression analysis revealed a positive correlation between the results of HS imaging and time to healing ($r=0.527$, $p=0.03$), with higher oxygenation again being associated with a worse outcome. This was observed in those either with ($p=0.009$) or without ($p=0.044$) clinical infection. No correlation was observed between healing and time to healing and either skin temperature or ABPI. 28 ulcers healed by 24 weeks (one patient died with ulcer unhealed). No correlation was observed between any baseline measure and either healing or time to healing at 24 weeks.

Conclusion: Although the result is superficially counterintuitive, the negative relationship between clinical outcome and oxygenation determined by HS imaging is explained by the fact that HS imaging is a measure of the intravascular oxygen saturation of haemoglobin. Neuropathy is associated with higher oxygen saturation of venous blood, with implied lower oxygenation of the extravascular tissues. In patients with abnormal microvasculature, HS imaging will therefore have an inverse relationship with both extravascular oxygenation and healing. HS imaging could prove a valuable predictor of 12 week outcome in newly presenting foot disease in diabetes.

Supported by: NIHR NEAT

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Neurotensin downregulates the pro-inflammatory properties of skin-dendritic cells and increases epidermal growth factor expression

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Background and aims: Diabetic foot ulcer is a painless and harmful disease that delays skin wound healing in diabetic patients. This illness is a consequence of differential causes, like neuropathy, impaired angiogenesis and decreased blood flow supply to the site of injury, cytokines deregulation, impaired expression of extracellular matrix (ECM) proteins and ECM remodeling by metalloproteinases. In the last three decades, neuropeptides received much attention by the scientific community, as their impairment, for instance by neuropathy, may induce delayed WH. Neuropeptides modulate skin cells function, particularly skin inflammation. As recent reports have revealed the neuropeptide neurotensin (NT) as an immune mediator in the Central Nervous System and the gastrointestinal tract and its effects in the skin have not been identified, in this study we investigated the effects of NT on signal transduction and on pro/anti-inflammatory function of skin dendritic cells in the presence/absence of the inflammatory stimuli lipopolysaccharide (LPS).

Materials and methods: Fetal-skin dendritic cells (FSDC) were maintained in IMDM medium (control) or incubated with 10 nM of NT alone or simultaneously with LPS for 5 to 60 min to analyze the activation of signaling pathways by WB and during 6 to 30 h to determine neurotensin receptors (NTRs), cytokines and growth factors expression by real time RT-PCR. FSDC viability was determined by the MTT assay while the cytoskeleton and nuclei morphology of FSDC was determined by immunostaining for actin and nuclei.

Results: We observed that FSDC constitutively express NTR1 and NTR2 and that LPS treatment diminishes NTR1 (-1.14 ± 0.55 , $n=3$), NTR2 (-1.88 ± 0.77 , $*p<0.05$, $n=3$), while expression of NT was increased by 3.75 ± 0.50 ($***p<0.0001$, $n=3$) relatively to control. In LPS stimulated cells, NT downregulated significantly the activation of the inflammatory signaling pathways NF- κ B and JNK, while the survival pathway ERK was upregulated. Neurotensin alone downregulated the expression of the cytokines IL-6 (-0.34 ± 0.26 , $n=3$), TNF- α (-1.10 ± 0.62 , $*p<0.05$, $n=3$), IL-10 (-1.77 ± 0.91 , $*p<0.05$, $n=3$) and the vascular endothelial growth factor (VEGF) (-0.29 ± 0.18 , $*p<0.05$, $n=4$) fold

relatively to control, while the epidermal growth factor (EGF) (1.45 ± 1.18 , $*p < 0.05$, $n=4$) was significantly upregulated. Simultaneous cell exposure to LPS and NT induced a similar cytokine profile to that one induced by NT alone. **Conclusion:** Overall, our results give new perspectives in the design of new therapies for skin diseases, like diabetic wound healing. Moreover, once NT levels are determined in skin pathologies and if NT is then defective, a treatment with NT may promote WH. Further similar studies should be performed both in other skin cells and in *in vivo* conditions, in order to disclose the potential benefic therapeutic role of NT on skin pathological conditions. *Supported by: SAU-MII/098567/2008 and EFSD/JDRF/Novo Nordisk grant SFRH/BD/60837/2009 (LM).*

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Histological features of acute Charcot neuroarthropathy in diabetes

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Background and aims: To provide a description of the histological features of bone affected by acute Charcot neuropathic osteoarthropathy (CN; acute Charcot foot).

Materials and methods: As part of a study of pro-inflammatory cytokines and signalling pathways in CN, needle biopsies were taken with full aseptic precautions, using a 14 g needle and yielding a sample approximately 2x8 mm. Nine of 17 patients newly presenting between Feb 2009 and Jan 2010 to a single specialist unit with active CN gave informed consent to undergo bone biopsy, under local anaesthesia where necessary. Control samples were examined from nine non-diabetic patients who were undergoing elective corrective surgery of the foot.

Results: There were no operative complications. There were two women and seven men with CN and mean age was 60.1 years (SD 8.31). The total median (range) duration of the active disease at the time of biopsy was estimated to be 10 (4–46) weeks. Histological examination revealed bone surface remodelling to be active in CN bone as assessed by osteoclast and osteoblast numbers. Some CN bone exhibited regions of chondroid material forming woven bone. The marrow space tended to show replacement of adipose tissue by loose spindle cells in the majority of CN specimens. In some cases this was vascular, resembling vascular granulation tissue. In other cases the marrow space was replaced by collagenous tissue with low vascularity. There was moderate hypercellularity of the periosteum in a number of biopsies. No sample showed features of an acute inflammatory reaction suggesting osteomyelitis. Overall, cases could be divided into three groups, but with some overlap in individual samples.

- Lamellar bone with active surface remodelling of trabeculae associated with microfractures. Marrow space replaced by loose spindle cells.
- Active remodelling of woven bone, with vascularity of the marrow space associated with increased vascularity, possibly suggesting a response to fracture.
- Sclerosis of bone characterised by broad lamellar trabeculae with collagenous replacement and a low vascularity of the marrow space.

Microbiological examination on the CN patients revealed no bacteria on Gram stain, although scanty polymorphs were observed in two cases. Coagulase negative *Staphylococcus aureus* was cultured from three cases and MSSA was cultured from a fourth. This fourth case had clinical and radiological evidence of osteomyelitis affecting the middle and distal phalanges of the third toe, but the bone sample was taken from the second metatarsal head. Histological examination of the biopsy from this last case revealed no features of acute osteomyelitis.

Conclusion: Given the occasional difficulty encountered in distinguishing between CN and osteomyelitis both clinically and on imaging, as well as their occasional concurrence, and given the increased emphasis currently placed on the use of histological and microbiological examination of bone to confirm the diagnosis of osteomyelitis of the foot in diabetes, it is important to document the results of histological and microbiological examination of bone in CN. This study also indicated that bone biopsy can be undertaken without apparent complication in this condition and examination of bone could therefore be used more frequently in order to explore the pathogenesis of this condition.

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The proinflammatory cytokines TNF- α and IL-6 modulate RANKL-mediated osteoclastic resorption *in vitro* in patients with acute Charcot osteoarthropathy

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Background and aims: The mechanisms of pathological bone resorption in acute Charcot osteoarthropathy are not fully understood. Recently, using a technique to generate functional human osteoclasts from peripheral blood mononuclear cells, we demonstrated that receptor activator of nuclear factor κ B ligand (RANKL) plays an important role as an activator of osteoclastic resorption in acute Charcot osteoarthropathy. However, it is not known whether RANKL-mediated osteoclastic resorption can be modulated by the proinflammatory cytokines TNF- α and IL-6. Thus the aim of this study was to investigate whether TNF- α and IL-6 can modulate RANKL-mediated osteoclastic activity *in vitro* in acute Charcot osteoarthropathy by using neutralising antibodies to TNF- α and IL-6.

Materials and methods: We have studied 6 patients with acute Charcot osteoarthropathy, 6 patients with diabetes and 4 healthy subjects with no history of diabetes. Peripheral blood mononuclear cells, which act as osteoclast precursors, were cultured *in vitro* on bovine disks in the presence of (1) macrophage-colony stimulating factor (M-CSF) and RANKL, (2) M-CSF, RANKL and neutralising antibody to TNF- α and (3) M-CSF, RANKL and neutralising antibody to IL-6. After 21 days in culture, the bovine discs were stained with toluidine blue and osteoclastic resorption was determined and expressed as percentage of the eroded surface.

Results: The newly generated osteoclasts in patients with acute Charcot osteoarthropathy exhibited increased resorbing activity in cultures with M-CSF and RANKL compared with diabetic and healthy controls ($p=0.004$). The percentage of bone resorption of the bovine disks was significantly greater in Charcot patients compared with diabetic patients ($35.9\% \pm 3.7$ versus $14.9\% \pm 4.4$, mean \pm SEM; $p=0.005$) and also greater in Charcot patients compared with healthy subjects ($35.9\% \pm 3.7$ versus $17.3\% \pm 3.5$, $p=0.007$). The addition of neutralising antibody to TNF- α to the cultures with M-CSF and RANKL led to a marked decrease in the osteoclastic resorption only in patients with Charcot osteoarthropathy (from $35.9\% \pm 3.7$ to $25.4\% \pm 12.4$, $p=0.009$) but not in patients with diabetes (from $14.9\% \pm 4.4$ to $14.9\% \pm 3.3$, $p=0.984$) nor in healthy subjects (from $17.3\% \pm 3.5$ to $17.1\% \pm 3.1$, $p=0.851$). The addition of neutralising antibody to IL-6 had a variable effect on the osteoclastic resorption in the three groups: it led to a significant decrease in osteoclastic resorption in patients with acute Charcot osteoarthropathy (from $35.9\% \pm 3.7$ to $29.3\% \pm 1.9$, $p=0.49$) and to a non-significant decrease in healthy controls (from $17.3\% \pm 3.5$ to $10.8\% \pm 32.3$, $p=0.261$). Unexpectedly, it led to a significant increase of bone resorption in patients with diabetes (from $14.9\% \pm 4.4$ to $22.7\% \pm 5.6$, $p=0.022$).

Conclusion: In acute Charcot osteoarthropathy, RANKL-mediated osteoclastic resorption is modulated *in vitro* by the proinflammatory cytokines TNF- α and IL-6.

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Local and systemic concentrations of pro-inflammatory cytokines, osteoprotegerin, sRANKL and bone turnover markers in acute Charcot foot and in controls

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Background and aims: It is thought that the pathogenesis of the acute Charcot foot (CN) is dependent on activation of the osteoprotegerin (OPG)/RANKL signalling pathway by pro-inflammatory cytokines and this stimulates increased bone breakdown. As part of a study designed to investigate these processes in greater detail, we sought to determine the differences that may exist between the concentrations of pro-inflammatory cytokines, OPG/sRANKL and bone turnover markers in the local and systemic circulation in order to determine the usefulness or otherwise of forearm vein sampling in future studies in this area.

Materials and methods: The study had ethical approval and patients with active CN managed at a single specialist centre between February 2009 and January 2010 gave informed consent to have samples of venous blood taken from the dorsal veins of both the affected and contralateral foot, as well as a forearm vein. Serum was aliquotted and stored at -20°C until assayed in batches for CRP, alkaline phosphatase (ALP), CTX, IL-6, IL-1, TNF- α , OPG and sRANKL. The results were compared with those of 17 non-diabetic control patients who gave consent to have sampling from a dorsal vein of the foot immediately prior to elective corrective surgery to the foot.

Results: 17 patients with CN (mean age 57.7, range 44–78 years; 5 T1DM, 12 T2DM) gave informed consent to participate. In patients with CN there was a significant difference ($p=0.003$, Chi square) between the concentrations of both IL-6 and CTX in local and systemic venous samples, with the highest concentrations being in blood from the affected foot (median IL-6: affected foot 7.6 pg/mL (95% CI 1.3–22.9), contralateral foot 5.8 pg/mL (8.6–12.1), arm 4.1 pg/mL (1.1–14.3); median CTX: affected foot 0.79 mcg/L (0.34–1.60), contralateral foot 0.5 mcg/L (0.14–1.10), arm 0.42 mcg/L (0.12–0.88)). No difference was observed between local and systemic samples for any other analyte in CN. When concentrations in the dorsal veins of the affected foot in CN and controls were compared, higher concentrations of CTX ($p=0.003$), ALP ($p=0.003$), OPG ($p=0.003$) and IL-6 ($p=0.000$) were observed in CN, but there were no differences in CRP, sRANKL and IL-1.

Conclusion: The present results confirm the expected increase in bone turnover markers in CN, and concentrations were highest in dorsal vein blood from the affected side. The increase in OPG may reflect the known association with diabetes. The absence of difference in concentrations from the local and systemic samples in patients with CN suggests that concentrations in forearm vein blood could be taken to reflect local concentrations of the analytes studied, with the single exception of IL-6. The marked elevation of IL-6 in dorsal vein blood of the affected foot in CN suggests that this cytokine may be particularly important in the pathogenesis of this disease.

OP 03 Nutrition and diet

13

High intake of protein and processed meat is associated with increased incidence of type 2 diabetes

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Background and aims: Diets high in protein have shown promising results on short-term weight reduction and glycemic control. However, understanding of how dietary macronutrient composition relates to long-term risk of obesity related chronic disease, such as type 2 diabetes is limited. We examined intakes of macronutrients, fiber and different food sources of protein in relation to incidence of type 2 diabetes.

Materials and methods: In total 27 140 individuals, 45–74 years, from the population based Malmö Diet and Cancer cohort were included. Dietary data was collected with a modified diet history method, including registration of cooked meals. During 12 years follow-up, 1709 incident type 2 diabetes cases were identified. We used Cox proportional hazard model to calculate hazard ratios of diabetes incidence in quintiles of dietary intakes with adjustments for several potential confounders.

Results: High protein intake was associated with increased risk of type 2 diabetes (hazard ratio [HR] = 1.37; 95% confidence interval [CI]: 1.17 to 1.61, P for trend < 0.001). In addition, 5 energy % increased consumption of protein at the expense of carbohydrates was associated with increased risk (HR = 1.17; 95% CI: 1.06 to 1.29). High intake of processed meat was also associated with increased risk (HR = 1.17; 95% C.I. 1.00 to 1.36, P for trend = 0.005), but the positive association between protein intake and type 2 diabetes remained significant after adjustment for intake of processed meat. Intake of fiber rich breads and cereals was inversely associated with type 2 diabetes (HR = 0.95; 95% CI: 0.92 to 0.98, P for trend = 0.005).

Conclusion: Results from this large population based study indicate that high protein intake increase the incidence of type 2 diabetes. Replacing protein with carbohydrates may be favorable, especially if fiber rich breads and cereals are chosen as carbohydrate sources.

Supported by: The Swedish Research Council

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Diets poor in fibre, vegetable protein and polyunsaturated fat are associated with inflammation and endothelial dysfunction in type 1 diabetes mellitus: the EURODIAB study

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Background and aims: To investigate the association between nutrient consumption and biomarkers of endothelial dysfunction (ED) and low-grade inflammation (LGI) in type 1 diabetes mellitus.

Materials and methods: We investigated 491 individuals. Nutrient consumption and lifestyle risk factors were measured in 1989 and 1997. Biomarkers of ED (von Willebrand factor, soluble vascular cell adhesion molecule-1 and soluble endothelial selectin) and LGI (C-reactive protein, interleukin-6 and tumour necrosis factor- α) were measured in 1997 and averaged into z scores. Nutrients were adjusted for total energy intake by the nutrient residual method. Data were analysed with generalised estimating equations. We report average 8-year differences in nutrient consumption per +1 standard deviation (SD) of 1997 ED or LGI z scores. Results are adjusted for sex, age, duration of diabetes, investigation centre, body mass index, energy intake, smoking behaviour and alcohol consumption, and other nutrients.

Results: A one SD elevation in endothelial dysfunction z score was associated with a diet over an 8-year period that was lower in fibre [β (95%CI): -0.09(-0.18;-0.004)], polyunsaturated fat [-0.18(-0.32;-0.05)] and vegetable protein [-0.10(-0.20;-0.001)]. For the low-grade inflammation z score the results showed a diet lower in fibre [-0.09(-0.17;-0.01)] and polyunsaturated fat [-0.14(-0.24;-0.03)], and higher in cholesterol [0.10(0.01;0.18)].

Conclusion: In type 1 diabetes mellitus, consumption of less fibre, polyunsaturated fat and vegetable protein and more cholesterol over an 8-year study period was associated with more ED and LGI. This supports the hypothesis that following the current dietary guidelines in type 1 diabetes mellitus may reduce cardiovascular disease risk by affecting ED and LGI favourably.

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Post-prandial high fat intake leads to acute exposure to circulating endotoxin in type 2 diabetes mellitus subjects

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Background and aims: Habitual post-prandial absorption of dietary fats increases systemic atherogenic lipoproteins and a pro-inflammatory response, a condition exacerbated by obesity, insulin resistance (IR) and type 2 diabetes mellitus (T2DM). In addition to the systemic influence of dietary lipids, gut derived bacteria [endotoxin or lipopolysaccharide (LPS)] is absorbed coupled to damaging lipoproteins as it crosses the mucosa. As such, atherogenic and inflammatory risk may arise through changing dietary lipoprotein patterns and increasing circulating LPS. The heightened postprandial systemic lipids and LPS could directly impact on adipose tissue as our previous *in vitro* studies suggest. However no analysis to date has examined whether metabolic state may alter lipid coupled circulating LPS post feeding. Therefore these studies sought to establish whether a SFA rich meal increases circulating endotoxin absorption and whether this is altered in disease states.

Materials and methods: All subjects (n=58) with and without T2DM were given a high-fat meal (meal: 75g fat, 5g carbohydrate, 6g protein) following an over-night fast [non-obese controls (NOC): age: 39.8(Mean±SE) 11.2yr, BMI: 25.3(mean±SD)3.3Kg/m²; n=10; Obese: age: 43.8±9.5yr; BMI: 33.3±2.6Kg/m²; n=15; impaired glucose tolerance (IGT): age: 41.7±3.3yr; BMI: 32.0±4.5Kg/m²; n=12; T2DM: age: 46.4±9.6yr; BMI: 30.1±5.2 Kg/m²; n=21]. Baseline (0 hr) and post-prandial sera (1–4 hr) were taken from subjects and LPS, inflammatory cytokines and lipid levels measured.

Results: Baseline circulating LPS was significantly higher in the T2DM, IGT, and obese subjects Vs NOC subjects (Baseline LPS: T2DM: 5.73(mean±SEM)0.85EU/mL*; Obese: 5.75±1.45EU/mL*; IGT: 5.83±0.45EU/mL* Vs NOC: 3.55±0.50EU/mL; *p<0.05). Comparatively baseline circulating LPS levels were 61.41% higher in T2DM subjects Vs NOC subjects. Ingesting a high-fat meal led to a significant rise in LPS levels in T2DM and IGT subjects over the post-prandial time period compared with baseline levels (T2DM: LPS 0 hr: 5.73±0.85 EU/mL Vs. T2DM 4 hr post-prandial (pp): 16.89±3.67EU/mL*; IGT: LPS 0 hr: 5.83±0.45 EU/mL Vs 4 hr pp: 7.69±0.72EU/mL*, p<0.05, **p<0.001); whilst only a trend was observed for obese or NOC subjects. These findings also showed that at 4hr post feed T2DM subjects exhibited a mean LPS level 118.5% higher than that of the NOC subjects. A strong positive correlation between increasing BMI and log LPS was also noted in the NOC and obese cohorts (r value= 0.501** and 0.301*, respectively; **p<0.001, *p<0.05), which was ultimately lost with either IGT or T2DM status.

Conclusion: In summary these studies highlighted that postprandial exposure to a high fat meal elevates circulating LPS irrespective of metabolic state, as early as 1 hr post meal. Post-feeding leads to a substantial increase in circulating LPS in IGT and T2DM subjects, suggesting that metabolic endotoxaemia is exacerbated post feeding as IR status increases. Our data highlight that T2DM subjects are exposed to as much as 118% more circulating LPS post-prandially, per high fat meal. Therefore a continual grazing routine will cumulatively promote their pathogenic condition more rapidly than other individuals due to the elevated exposure to endotoxin.

Supported by: BHF

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Plasma insulin and inflammatory markers prior to weight loss can predict dietary responders

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Background and aims: The ability to identify obese subjects who will lose weight in response to energy restriction is an important strategy in obesity clinical care.

Materials and methods: Fifty obese/overweight subjects were submitted to an energy restricted high protein diet (35% protein, 25% fat and 40% carbohydrate) and rich in fibers for 6 weeks followed by a 6 weeks-maintenance diet. Body composition, plasma metabolic and inflammatory markers as well as adipose tissue inflammation marker (HAM56) were measured at baseline, 6 weeks after diet intervention and the end of the study. Baecke physical activity scores and psychological scores (BECK questionnaire, three factor eating questionnaire) were also evaluated. Gut microbiota were profiled from faecal samples by qPCR in all the subjects.

Results: Based on their trajectories of weight loss during the study, three subject clusters were identified. Cluster A (n=17) and Cluster B (n=15) lost more weight during the diet period, however during the stabilization phase cluster A continued to lose weight, whereas cluster B remained stable. Cluster C (n=17) lost less and rapidly regained weight during the stabilization. At baseline, subjects in cluster C had the highest plasma insulin (P=0.01), IL-6 (P=0.05) and adipose tissue inflammatory marker (HAM56, P=0.03). Intriguingly subjects in cluster C had the highest level of Lactobacillus/Leuconostoc/Pediococcus group before diet. During the dietary program, subjects in cluster C consumed more starchy foods and less protein and raw vegetables. Spearman correlations revealed positive relationships between weight regain after diet and HOMA-IR (P=0.0002, rs=0.5), inflammatory markers (leucocytes numbers: P=0.05, rs=0.3; Neutrophils: P=0.05, rs=0.3; IL-6: P=0.002, rs=0.43) as well as the number of Lactobacillus/Leuconostoc/Pediococcus group (p=0.005, rs=0.4) at baseline. Bayesian network (BN) was performed for prediction of the 3 clusters by using the data prior to the weight-loss dietary program. According to the learned structure of BN, the levels of 4 biomarkers (plasma insulin, IL-6, leucocytes numbers and adipose tissue HAM56) at baseline were sufficient to characterize the distribution of the 3 clusters. The prediction of clusters was 75.5% (37 among 49 subjects).

Conclusion: We concluded that individual responses to hypocaloric high protein dietary program could be predicted by plasma insulin, IL-6, leucocytes numbers and adipose tissue HAM56 levels prior to diet.

Clinical Trial Registration Number: CRIC NCT 0047658

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Thorough chewing stimulates postprandial increases of plasma GLP-1 and peptide YY in obese subjects

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Background and aims: Glucagon like peptide (GLP)-1 and peptide YY (PYY) are secreted from intestinal L cells, and plasma levels of both hormones rise after a meal. GLP-1 stimulates glucose-dependent insulin secretion. PYY decreases appetite and reduces food intake by acting on receptors in the hypothalamus. GLP-1 also reduces food intake. Therefore, GLP-1 and PYY seem important to control plasma glucose and triglyceride (TG) levels and body weight. On the other hand, it has been conventionally thought that "chewing well" i.e. "thorough chewing" is good for health. Much remains unknown on the relationship between thorough chewing and GLP-1 and PYY which are secreted from intestine after a meal. The aim of this study is to investigate effects of thorough chewing on postprandial levels of plasma GLP-1 and PYY in obese subjects.

Materials and methods: Nine obese subjects were recruited. They were not diabetic. Obesity was defined as BMI above 25 according to Japanese criteria.

Mean fasting plasma glucose level was 99 mg/dl. Mean age was 40.7 years and mean BMI was 27.2. The subjects were given the test meal early in the morning after 12h fasting. They ate it for 20 minutes and chewed each mouthful 5 times (5 times chewing). On the other day the subjects ate it for 20 minutes and chewed each mouthful 30 times (30 times chewing, i.e. thorough chewing). Plasma GLP-1 and PYY were measured before and 1h after ingestion of the test meal. The test meal consisted of bread, margarine, a boiled egg, steamed vegetables, a banana, and milk. Total calories were 630 kcal, with 16% protein, 32% fat, and 52% carbohydrate. Plasma PYY was measured as PYY(3-36) using an enzyme immunoassay kit. Plasma GLP-1 was measured as GLP-1(7-36) using ELISA kit.

Results: Plasma mean PYY levels with 5 times chewing tended to increase from 35.8 pg/ml (before a meal) to 41.3 pg/ml (after a meal). Plasma PYY levels with 30 times chewing significantly increased from 35.7 pg/ml (before a meal) to 65.9 pg/ml (after a meal). Postprandial PYY level with 30 times chewing was significantly higher than with 5 times chewing. Plasma mean GLP-1 levels with 5 times chewing significantly increased from 4.6 pmol/l (before a meal) to 16.9 pmol/l (after a meal). Plasma GLP-1 levels with 30 times chewing significantly increased from 5.1 pmol/l (before a meal) to 29.3 pmol/l (after a meal). Postprandial GLP-1 level with 30 times chewing was significantly higher than with 5 times chewing.

Conclusions: This is the first report that thorough chewing stimulates postprandial increases of plasma GLP-1 and PYY in obese subjects. Thorough chewing may be clinically effective in obese subjects.

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Diabetes specific nutrition improves post-prandial glycaemia and GLP-1 with similar appetitive responses compared to a typical healthful breakfast in persons with type 2 diabetes

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Background and aims: For people with diabetes mellitus (DM), the frequency and/or composition of the morning meal may be especially important as the hormonal and metabolic perturbations associated with the disease contribute significantly to poor glucose control and weight gain. Few well-controlled studies have evaluated the effects of breakfast skipping or meal composition on metabolic control, post-prandial glucagon-like peptide-1 (GLP-1) release and appetite in this patient population. This study evaluated the impact of skipping breakfast compared to eating two convenient and healthful breakfast options on post-prandial blood glucose, GLP-1 and appetite.

Materials and methods: This was a randomized, controlled, non-blinded, three- treatment, crossover study conducted at two study sites. Subjects (n=32) had type 2 DM controlled by medication. On the morning of each study period, fasted subjects consumed each test meal in random order: the three nutritional interventions administered were (1) no breakfast (NB), (2) a typical whole food breakfast meal consisting of instant oatmeal (TB) and (3) calorically-matched diabetes-specific liquid nutritional formula (DSN). The energy provided from these two caloric interventions was: 200 kcals (8 g protein, 36 g CHO, 6 g fiber, 4 g fat) and 216 kcals (10 g protein, 29 g CHO, 2 g fiber and 8 g fat), respectively. Blood samples for measurement of plasma glucose, insulin, GLP-1 were collected at baseline and after consumption of the test meal over 180 minutes. Appetitive responses were assessed immediately after each blood collection by 100mm visual analog line scales appropriately anchored by modality.

Results: Post-prandial plasma glucose positive area under the curve (AUC) was significantly reduced by 38% after consumption of the DSN (Least Squares Mean (LSM) \pm Standard Error (SE); 4119 ± 461 (mg/dL)*min; repeated measures ANOVA; $p < 0.0001$) as compared to TB (7531 ± 461). The consumption of NB had the smallest mean positive AUC (437 ± 460) of all interventions. The LSM from post-prandial plasma peak glucose values were 142 ± 7 for NB, 214 ± 7 TB and 178 ± 8 for DSN and each pair wise comparison was significantly different from one another (each comparison $p < 0.0001$). GLP-1 post-prandial AUC was significantly elevated after the consumption of DSN by 246% compared to TB (Signed rank test; $p < 0.0001$; medians, 420 vs. 226) and 995% compared to NB ($p < 0.0001$; 420 vs. 111). Subjective hunger rating positive AUC was significantly elevated after NB (mean \pm SE; 2658 ± 513) compared to both the TB (820 ± 249 ; $p = 0.008$) and DSN (1005 ± 314 ; $p < 0.0001$) interventions. Subjective fullness ratings positive AUC were significantly lower from NB (mean \pm SE; 1013 ± 337) compared to TB (4047 ± 571 ; $p < 0.0001$) and DSN (3402 ± 542 ; $p < 0.0001$). No differences in hunger or fullness were observed between the DSN and TB interventions.

Conclusion: DSN had improved overall plasma glucose control and was the only caloric intervention that had a mean peak post-prandial blood glucose below the recommended value of <180 mg/dL compared to TB. Skipping breakfast was associated with excessive appetitive responses which were not observed with TB and DSN. Additionally, the DSN resulted in elevated postprandial GLP-1, which further supports the beneficial impact of nutritional interventions.

Clinical Trial Registration Number: NCT01324921

OP 04 Brown adipose tissue and mitochondria

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Pilot study: activity of brown adipose tissue in healthy men during fasting and feeding

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Background and aims: Brown adipose tissue (BAT), long thought to dwindle after infancy, is present and physiologically active in many adults. Its role in human metabolism is poorly understood. The aim of this pilot study was to examine the effects of feeding, a situation of physiologically raised insulin concentrations, on BAT activity.

Materials and methods: We included 10 healthy lean male volunteers (18 to 35 years; body mass index 20–24 kg/m²). BAT activity was visualised by glucose uptake in BAT using ¹⁸F-FDG PET-CT during cold exposure. The subjects had two subsequent PET-CT scans, separated by at least two weeks. The first PET-CT was performed after an overnight fast; the second PET-CT was performed after a standardised meal (545 Kcal, composition: 34 gram fat, 37 gram carbohydrates and 23 gram protein).

Results: BAT activity was observed in 6 out of 10 volunteers. These subjects were found to have a higher standardised uptake value (SUVmax) in the fasting state; mean (SD) 13.4 (9.4) compared with the non-fasting state: 6.6 (4.9) ($P=0.03$). Mean (SD) fasting glucose was 5.1 (0.2) mmol/L and mean insulin levels were 30.0 (17.9) pmol/L. On the second study day, one hour after the meal, mean (SD) glucose was 4.0 (0.8) mmol/L and mean insulin levels were 106.3 (56.0) pmol/L.

Conclusion: Glucose uptake in BAT in humans is more pronounced in the fasting state. The lower SUVmax in the non-fasting state may be explained by a steal phenomenon, as a result of increased glucose uptake in muscle.

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Expression of brown adipose tissue markers in different human adipose tissue depots

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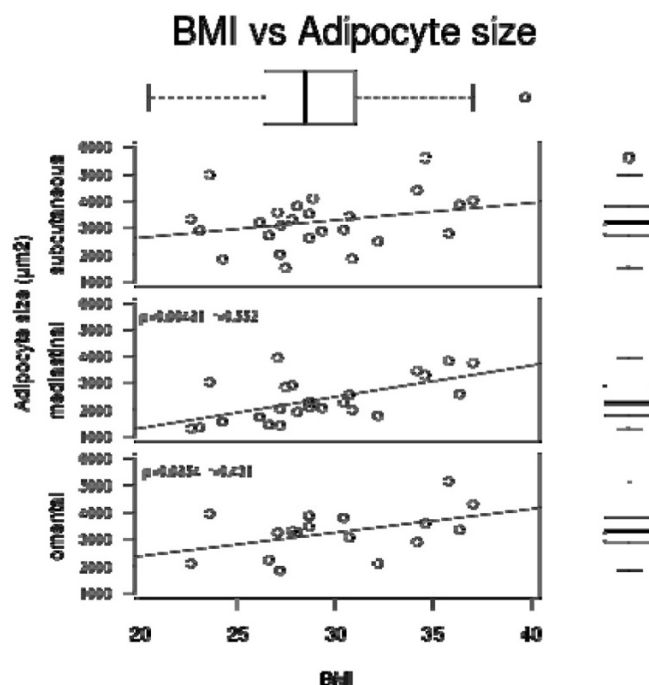
Background and aims: Recent findings of brown adipose tissue (BAT) in adult humans using positron emission tomography scanning prove that BAT is still present in adult humans, at least when activated by cold exposure. While these studies focused mainly on the more common BAT depots around the neck in the cervical-supraclavicular and/or interscapular region, we aim to unveil the possible BAT characteristics in another potentially BAT depot in mediastinum (outside the heart cavity).

Materials and methods: Adipose tissue biopsies from subcutaneous (anterior upper thoracic), mediastinal and omental depots were collected from at least 30 patients (age 37–85, BMI 21–40). The mRNA expression levels of two known brown adipose tissue genes - uncoupling protein 1 (UCP1) and PR domain containing 16 (PRDM16) - were measured by TaqMan expression assay. The protein levels of UCP1 were examined by immunoblot and immunohistochemistry. Adipocyte cell sizes were computed using ImageJ software on paraffin-embedded sections.

Results: The adipocytes in mediastinal depot were significantly smaller than those in the subcutaneous and the omental depots. Moreover, adipocyte size in the mediastinal depot was positively correlated to the subjects' BMI, while a tendency of such correlation was found between omental adipocyte size and BMI. We observed mRNA expression of the BAT markers in all three depots. The expression levels were significantly higher in the mediastinal than the upper chest subcutaneous depot and the difference was more pronounced in the expression of UCP1 than that of PRDM16. Immunohistochemistry analysis examining the cell type and localization of UCP1-antibody positivity was also performed.

Conclusion: These results suggest that BAT properties might present in the adult human mediastinal adipose tissue. The difference in expression levels of

BAT markers across depots might indicate the degree of "brown-ness/whiteness" of the specific depot, which deserves further investigation to dissect its biological significance, such as in relation to insulin sensitivity.



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New insulin sensitizers produce differentiation of brown-like adipose cells from a subcutaneous fat depot and increase secretion of adiponectin *in vitro*

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Background and aims: Subcutaneous adipose depots have been suggested to play an important role in promoting insulin sensitivity in rodents and man. The subcutaneous adipose depots are also a major source of adiponectin, an adipokine with salutary hormone sensitizing effects on insulin target tissues. New insulin sensitizers that do not bind to or activate PPAR γ at micromolar concentrations [PPAR γ -sparing thiazolidinediones (PSTZDs)] have been used to evaluate the possible influence of the PSTZDs on subcutaneous adipose tissue.

Materials and methods: Progenitor cells from axillary adipose depots were isolated from CD-1 mice and placed in tissue culture under defined conditions. Treatment of these cells with PSTZDs, including clinical candidate MSDC-0160, was used to examine PPAR γ independent effects on both differentiation and the associated secretion of adiponectin.

Results: Addition of the PSTZDs such as the clinical candidate MSDC-0160, elicited a time- and dose-dependent differentiation of the progenitor cells which exhibited a brown-like morphology with multilocular fat droplets and intense oil red O staining. Assessment of expression of the mitochondrial uncoupling protein (UCP1) revealed that 3 μ M MSDC-0160 induced a greater than 2000-fold increase in UCP1 mRNA and a 20-fold increase in UCP1 protein within 7 days. Treatment with micromolar MSDC-0160 also produced a two-fold increase in mitochondrial biogenesis as measured by the mitochondrial marker, citrate synthase activity. These effects were not attenuated by the potent PPAR γ antagonists, T0070907 and GW9662, confirming the PPAR-sparing nature of the drug action. Adiponectin expression in the cultures was also studied as a function of drug treatment. MSDC-0160 treatment produced a more than 15-fold increase in adiponectin mRNA within 6 days of drug treatment. Under these conditions, the secretion of adiponectin into the medium was also increased in a PPAR γ -independent and time-dependent manner with maximal increases in secreted protein after 4 days of drug treatment in response to micromolar levels of compound.

Conclusion: In conclusion, we report that the PsTZDs elicit differentiation of progenitor cells from axillary adipose depots into brown-like adipose cells that express UCP1 message and protein in a process that includes increased mitochondrial biogenesis. Importantly, in contrast to the effects on brown adipose cells from the interscapular pad, the cells from axillary adipose depots also secreted adiponectin in response to the PsTZDs. Since activation of PPAR γ is known to be responsible for dose limiting side effects of currently available insulin sensitizers, these results have important mechanistic implications for future therapies of type 2 diabetes and provide a framework for defining the key biochemical pathways involved in the action of PsTZDs, including production and secretion of adiponectin.

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Obesity mediates mitochondrial dysfunction in islets, liver and adipose tissue

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Background and aims: The cellular life cycle of mitochondria is mediated by fusion and fission events. Mitofusin 1 and 2 (Mfn1 and Mfn2) and optic atrophy 1 (Opa1) are proteins controlling mitochondrial fusion. Mitochondrial network elongation is terminated by fission, a process regulated through the fission protein 1 (Fis1) and the dynamin related protein 1 (Drp1). Fis1 and Drp1 play specific roles in the assembly of the fission complex and cannot replace each other. It was shown in recent studies, that high concentrations of free fatty acids evoke defects in the mitochondrial network and the integrity of the respiratory chain. Free fatty acids are elevated during obesity and contribute to the development of type 2 diabetes mellitus. Therefore the aim of this study was to analyze mitochondrial function and dynamics in obese ob/ob mice compared with normal weight control mice.

Materials and methods: Liver, muscle, pancreas and adipose tissue were taken from ob/ob (B6.V-leobob) and control mice. Islets were isolated by collagenase digestion. Gene expression of Fis1, Drp1, Opa1, Mfn1 and Mfn2 was determined by quantitative Real-Time PCR analyses. Protein production was defined by western blots and immunofluorescence analyses. The mitochondrial network was analyzed using MitoTracker Deep Red staining.

Results: All analyzed genes involved in fusion and fission were down regulated in islets of obese ob/ob mice compared with control mice. In addition to the overall reduced mitochondrial life cycle, Fis1 gene expression and protein production was significantly reduced. Only minor Fis1 immunofluorescence was detectable in insulin immunofluorescence positive cells of pancreas sections of ob/ob mice compared to control mice. Also in liver Fis1 gene expression was significantly reduced in ob/ob mice compared to control. However, in contrast to islets, Drp1 was up regulated in the liver of ob/ob mice. Further analyses of the mitochondrial network structure through fluorescence microscopy revealed an inhomogeneous pattern with concomitant elongated and fragmented mitochondria. A homogeneous mitochondrial network was present in liver of control mice. Furthermore gene and protein expression of Fis1 was significant reduced in adipose tissue of ob/ob mice in comparison to control, whereas in muscle Fis1 production was marginal changed. However, Opa1 which was least affected in liver and adipose tissue was significant down regulated in muscle. Interestingly reduced expression of complex I and 2 of the respiratory chain and the ATP synthase (complex V) was observed in liver and adipose tissue but not in muscle of ob/ob mice compared with control mice.

Conclusion: The mitochondrial network structure and the integrity of the respiratory chain are altered in islets, liver and adipose tissue of obese mice. Reduced mitochondrial dynamic and especially Fis1 down regulation were elucidated in these tissues compared with control mice. In conclusion, our study provides in vivo evidence that obesity mediates mitochondrial dysfunction in insulin secreting and insulin responsive cells and thus, contributes to the development of type 2 diabetes mellitus.

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Mitochondrial bioenergetics changes are induced by metabolic endotoxaemia contributing to mitochondrial stress and dysfunction in human adipocytes

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Background and aims: Metabolic endotoxaemia is considered to contribute to an inflammatory state and insulin resistance (IR) in obese and type 2 diabetes mellitus (T2DM) subjects. Endotoxaemia arises through gut derived bacteria, lipopolysaccharide (LPS) and its action on innate immune response from adipose tissue (AT) exacerbated in obesity. Since we understand patients with severe IR have increased muscle derived mitochondrial dysfunction, we investigated whether LPS may, in part, influence mitochondrial function and efficiency in human AT/adipocytes. Therefore, we examined effects of LPS on mitochondrial related genes in human adipocytes and assessed the bioenergetic response to the influence of LPS on mitochondrial efficiency using Seahorse XF24 extracellular flux analyser. XF24 system enables measurements of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in real-time.

Methods and materials: AT (Abd Sc AT; BMI: 20.1–33.9 kg/m², age: 29–48 yr; n=35) was taken from subjects undergoing elective surgery with ethical approval. Differentiated pre-adipocytes were treated (0–6 hr) with LPS 100 ng/ml or 1000 ng/ml and mitochondrial gene expression assessed by qRT-PCR. OCR and ECAR were measured via the XF24 in a human adipocyte cell line (CHUB-S7) under treatment with LPS 100 ng/ml and 1000 ng/ml, in addition to drugs influencing the respiratory chain (oligomycin, FCCP and rotenone/antimycin A).

Results: Acute LPS treatment (0–6 hr) led to up-regulation of PGC1 α (p<0.05), COX3 (p<0.05), UCP 3 (p<0.001) and NRF1 (p<0.01) compared with untreated controls. For the XF24 extracellular flux analyser differentiated CHUB-S7 cells were seeded at 2.5x10⁴ cells/well and treated with LPS, an ATP Coupler (Oligomycin), ETC Accelerator (FCCP) and a combination of Antimycin and Rotenone (inhibitors of mitochondrial respiration). LPS 1000 ng/ml compromised glycolysis and mitochondrial respiration (vs LPS 100 ng/ml) as measured by the OCR (pmole O₂/min):ECAR (mpH/min) ratio [Control (no stressor): 10.04:1; Stress control: 7.68:1; LPS (100 ng/ml): 13.9:1; LPS (1000 ng/ml): 13.63:1]. Following injection of a mitochondrial uncoupler (FCCP) onto the treated cells, respiration rate (OCR) was increased by the stressor control (201 pmole O₂/min), 100 ng/ml LPS (305.4 pmole O₂/min) and 1000 ng/ml LPS (254 pmole O₂/min). However, there was a concentration dependent reduction (16.98%↓; p<0.01) in respiration rate with increasing LPS concentration leading to increased stress.

Conclusion: In summary our current data suggest that metabolic endotoxaemia may contribute to mitochondrial dysfunction in human AT providing a further insult in the pathogenesis of obesity mediated T2DM. The use of the dynamic real-time extracellular flux analyser, highlighted the detrimental impact of LPS on mitochondrial function and metabolism. These current data show LPS induces and exhausts the respiratory reserve capacity of the uncoupled mitochondria within adipocytes leading to a dose dependent increase in mitochondrial stress. The mRNA data also affirms that LPS induced mitochondrial stress. The OCR:ECAR ratio data shows that LPS compromises glycolysis as well as mitochondrial respiration highlighting the damaging effects of endotoxaemia.

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Molecular mechanisms of rosiglitazone-induced mitochondrial biogenesis in adipose tissue

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Background and aims: Several studies have shown a strong association between insulin resistance and decreased mitochondrial mass and oxidative capacity in tissues of insulin resistant patients, leading to the hypothesis that mitochondrial dysfunction may be an underlying cause for the development of type 2 diabetes. Mitochondrial mass is primarily modulated at the transcriptional level by several transcription factors whose activity is coordinated by members of the PGC-1 family of co-activators. Interestingly, decreased lev-

els of PGC-1 α have been observed in muscle and white adipose tissue (WAT) of insulin resistant patients, suggesting that reduced PGC-1 α expression, and the consequent impairment of mitochondrial function, could contribute to the onset of type 2 diabetes. Conversely, treatment with thiazolidinediones, a class of PPAR γ agonists widely used as anti-diabetic agents, increases PGC-1 α levels and promotes mitochondrial mass in WAT. Therefore, it has been proposed that thiazolidinediones may exert their beneficial effect on insulin sensitivity in part by promoting mitochondrial biogenesis in WAT through PGC-1 α . Here, we address the function of adipose PGC-1 α in regulating basal and rosiglitazone-induced mitochondrial biogenesis in WAT, as well as its effect on whole body energy balance and insulin sensitivity.

Materials and methods: To study the role of PGC-1 α on mitochondrial biogenesis in WAT, we have generated a mouse model in which the *pargc1a* gene is ablated by homologous recombination specifically in adipose tissue (PGC1 α -FAT-KO mice). Male PGC1 α -FAT-KO mice and Wild type (Wt) littermates were fed a high fat diet to induce insulin resistance and then treated with either vehicle or 10 mg/Kg rosiglitazone twice daily for 15 days.

Results: Mice lacking PGC-1 α expression in WAT were morphologically normal, although they exhibited a tendency towards a decreased body weight. Despite the well-known role of PGC-1 α in regulating mitochondrial gene expression, no differences in the expression of genes encoding for proteins of the oxphos system or involved in fatty acid oxidation were found in WAT between Wt and PGC1 α -FAT-KO mice. In Wt, rosiglitazone induced the expression of PGC-1 α by 2-fold, as well as the expression of mitochondrial genes (COXII, COXIV, ATP5b, MCAD). Consistent with an increase in mitochondrial gene expression, rosiglitazone induced mitochondrial mass and citrate synthase activity in Wt. Surprisingly, despite the lack of PGC-1 α , rosiglitazone induced mitochondrial gene expression and citrate synthase activity in PGC1 α -FAT-KO mice to levels comparable of those in Wt. No difference in insulin sensitivity was observed between WT and PGC1 α -FAT-KO. However, we found that the lack of PGC-1 α prevented the induction by rosiglitazone of UCP-1 and other brown adipocyte-specific genes, such as CIDEA or CPT1b.

Conclusion: Our results show that PGC-1 α is dispensable for basal and rosiglitazone-induced mitochondrial biogenesis in WAT, and that lack of PGC-1 α in adipose tissues does not affect whole body insulin sensitivity. However, PGC-1 α is required for the rosiglitazone-induced expression of UCP-1 and other brown adipocyte-specific genes in WAT, suggesting that PGC-1 α plays a role in the recruitment of brown adipocytes by rosiglitazone in WAT.

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OP 05 Genetics of type 1 diabetes

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PTPN2, a candidate gene for type 1 diabetes, modulates pancreatic beta cell apoptosis via regulation of the BH3-only protein Bim

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Background and aims: In recent years genome-wide association studies allowed the identification of several robust associations between specific chromosomal *loci* and type 1 diabetes (T1D). The pathophysiological mechanisms by which most candidate genes predispose to T1D development, however, remain unclear. PTPN2, located on chromosome 18p11, is a candidate gene for T1D expressed in both immune cells and pancreatic beta cells. PTPN2 modulates interferon (IFN) γ - and double stranded (ds)RNA-induced beta cell apoptosis. We presently evaluated the mechanisms by which PTPN2 triggers pancreatic beta cell apoptosis following exposure to type I and II IFNs.

Materials and methods: INS-1E and primary FACS-purified rat beta cells were transfected with two different small interfering RNAs targeting PTPN2 (inhibition of >70 %) and subsequently exposed to type I and II IFNs. Viability assays were performed by Hoechst/Propidium Iodide staining and confirmed by Western blotting analysis of caspases 9 and 3 activation. ISRE and GAS reporter activity were measured in PTPN2-silenced beta cells after IFN α or IFN γ treatment. The mitochondrial pathways of cell death were analyzed by immunofluorescence and/or Western blot. Expression of DP5, PUMA and Bim was studied by real time RT-PCR and Bim phosphorylation was detected by Western blot. Moreover, PTPN2 and Bim were silenced in a double-knockdown approach and viability tests were performed after IFNs treatment.

Results: PTPN2 knockdown significantly exacerbated type I IFN-induced apoptosis in INS-1E and primary rat beta cells (3.8- and 2.3-fold increase respectively; $p < 0.001$). Silencing of PTPN2 increased ISRE and GAS reporter activity after IFN treatment (1.7- and 2.0-fold induction respectively; $p < 0.001$). Double knockdown of PTPN2 and STAT1 reversed the pro-apoptotic effect of PTPN2 inhibition. Furthermore, PTPN2 silencing induced BAX translocation to the mitochondria, cytochrome c release to the cytosol and caspases 9 and 3 activation after exposure to type I and II IFNs, indicating activation of the intrinsic mitochondrial pathway of apoptosis. Although mRNA expression of DP5 and PUMA was not affected by PTPN2 knockdown, Bim mRNA expression was modestly increased at 16h or 24h of IFN α or IFN γ treatment. Importantly, Bim was hyperphosphorylated at Serine 65 in PTPN2-silenced beta cells. Bim knockdown reverted the pro-apoptotic effect of PTPN2 inactivation in INS-1E cells and primary rat beta cells by 56-78% and 59-63% respectively ($p < 0.001$).

Conclusion: We demonstrate that local IFN production may interact with a genetic factor (PTPN2) to induce aberrant pro-apoptotic activity of the BH3-only protein Bim, resulting in increased beta cell apoptosis via the mitochondrial pathway. This is the first indication of a direct interaction between a candidate gene for T1D and the activation of a specific downstream pro-apoptotic pathway of beta cells.

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Variants in PTPN22 and HLA-DQ haplotypes are associated with presence of autoantibodies but not with progression to diabetes in non-diabetic adults

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Background and aims: The significance of positivity for GAD-antibodies (GADA) at population level is unclear. We have previously shown that GADA and family history of type 1 diabetes increase the risk of non-insulin dependent diabetes. The aim for this study was to assess whether gene variants predisposing to type 1 diabetes were associated with GADA, decreased insulin secretion, or development of diabetes in a non-diabetic adult population. Furthermore, we investigated whether these variants explained the association of type 1 diabetes family history with progression to non-insulin dependent diabetes.

Materials and methods: Polymorphisms in *INS* (rs689), *PTPN22* (rs2476601), *CTLA4* (rs3087243) and the *HLA-DQA1-DQB1* region (rs2187668 and rs7454108 tagging HLA-DQ2.5 and HLA-DQ8, respectively) were genotyped in the Botnia prospective study (n=2764). The participants were followed for a median time of 8.1 years.

Results: At baseline HLA-DQ high risk (DQ2.5/DQ8, DQ8/DQ8, DQ8/DQX) and *PTPN22* CT/TT genotypes were associated with GADA (GADA- vs. GADA+; HLA high risk: 22.6% vs. 31.2%, $p=0.002$; *PTPN22* CT/TT: 19.9% vs. 28.0%, $p=0.003$). This association was dependent on GADA concentration (GADA- vs. GADA middle quartiles vs. GADA highest quartile; HLA-DQ2.5/DQ8: 3.2% vs. 3.8% vs. 15.4%, $p<0.000001$; *PTPN22* CT/TT: 19.9% vs. 26.5% vs. 32.3%, $p=0.007$). HLA-DQ2.5/DQ8 was especially associated with GADA in subjects aged < 40 yrs. Furthermore, association was observed between HLA-DQ high risk and *PTPN22* CT/TT genotypes and family history of type 1 diabetes irrespective of GADA (GADA-: $p<0.000001$ and $p=0.03$; GADA+: $p=0.002$ and $p=0.03$, respectively). At baseline subjects having a high genetic risk score showed increased insulin sensitivity [median (interquartile range) Insulin Sensitivity Index: 142 (111) vs. 144 (118) vs. 157 (127), $p=0.01$; HOMA_{IR}: 1.06 (0.90) vs. 1.10 (0.88) vs. 0.97 (0.79), $p=0.02$] and decreased insulin secretion [Insulinogenic index: 13.27 (16.27) vs. 12.69 (15.27) vs. 10.98 (13.06), $p=0.02$; Corrected Insulin Response: 115 (125) vs. 106 (124) vs. 97 (107), $p=0.082$, df2]. At follow-up subjects having a high genetic risk score were less able to compensate for the increased insulin demand. The investigated gene variants did not individually or as a combined genetic risk score predict development of diabetes, neither did the variants explain the previously observed association between family history of type 1 diabetes and development of type 2 diabetes.

Conclusion: Variants in HLA and *PTPN22* were associated with GADA and family history of type 1 diabetes in a non-diabetic population. None of the investigated variants was associated with development of diabetes or explained the association between family history of type 1 diabetes and progression to type 2 diabetes.

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The type 1 diabetes risk variant rs3825932 correlates with cathepsin H expression and disease progression in newly diagnosed children

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Background and aims: Recent studies have identified association of over 50 chromosomal regions with type 1 diabetes. Here, we focus on the region 15q25 containing four candidate genes (*ADAMTS7*, *CTSH*, *MORF4L1* and *RASGRF1*). The risk-associated single nucleotide polymorphism (SNP) rs3825932 in this region has previously been reported to affect the gene expression level of *CTSH* encoding the lysosomal protease cathepsin H. The aim was to examine how rs3825932 affects *CTSH* expression as well as disease mechanisms and progression. Since *CTSH* has been implicated in endoplasmic reticulum (ER) stress, an effect of rs3825932 on ER stress was examined as well.

Materials and methods: The genotype effect of rs3825932 on the gene and protein expression level of *CTSH* was investigated in 59 and 16 HapMap CEU lymphoblastoid cell lines (LCLs), respectively. The effect of the rs3825932 CC and TT genotypes was examined on the gene expression levels of six ER stress response genes (*ATF6*, *EDEM1*, *HERPUD1*, *HSPA5*, *SELS* and *XBP1*) using 14 HapMap LCLs. Genotype effects on gene and protein expression were evaluated using two-way analysis of variance and comparison between specific genotypes was done using t-test. Further, the gene expression level of *CTSH* was examined in human pancreatic islet preparations (n=13) in response to cytokine exposure and expression changes between untreated and cytokine-treated preparations were evaluated using paired t-test. Additionally, rs3825932 was examined for genotype effects on residual β -cell function and glycemic control in newly diagnosed type 1 diabetic children (n=257) using multiple regression models.

Results: We identified a significant allele-dosis effect of rs3825932 on *CTSH* mRNA ($p=1.3 \times 10^{-7}$) as well as protein ($p=0.014$) level in HapMap LCLs and found the risk-associated major C allele correlated with higher expression levels. Furthermore, the C allele of rs3825932 was correlated with a higher expression level of *XBP1* that mediates transcriptional induction during ER stress, compared to the T allele ($p<0.05$). In human pancreatic islets,

the expression of *CTSH* was decreased in response to cytokine stimulation ($p<0.01$). In children with newly diagnosed type 1 diabetes, the TT genotype of rs3825932 was associated with a higher daily insulin dose ($p<0.01$) and a more rapid disease progression.

Conclusion: Our study strengthens *CTSH* as a type 1 diabetes risk candidate gene. Further investigations will elucidate the functional implication of *CTSH* in type 1 diabetes.

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The effect of GWAS risk variants on disease progression within the first year after disease onset in children with type 1 diabetes

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Background and aims: Genome-wide association scans (GWAS) in type 1 diabetes (T1D) have highlighted predominantly classical immune-regulatory pathways as being involved in the pathogenesis of disease. However, it appears that T1D does not develop unless initial β -cell damage has taken place. We hypothesize that candidate genes linked to the genetic risk variants may be involved in disease mechanisms within the β -cells rendering them less able to withstand an attack from the immune system. The main objective of this study was to investigate whether genetic risk variants identified in GWAS of T1D could be associated with measures of disease progression and metabolic control in children within the first year after T1D onset.

Materials and methods: Expression profiles for pin-pointed candidate genes within 42 associated loci were investigated in nine human islet preparations (TaqMan, Applied Biosystems) and compared between the untreated control and cytokine treated (IL1- β , IFN- γ , TNF- α for 48h) condition using paired t-tests. Candidate genes with significant regulation were prioritized for analysis of their potential role in disease progression in an international prospective cohort of newly diagnosed children followed for one year after diagnosis. SNPs corresponding to the candidate genes were genotyped in 257 children (KASPar, KBioscience), 126 girls and 131 boys with mean age at clinical onset 9.1 ± 3.7 years. Using linear regression the effect of each SNP was tested on stimulated C-peptide levels 1, 6 and 12 months after diagnosis. Insulin dose-adjusted HbA1c (IDAA1c) defined as actual HbA1c + (4 x insulin dose (U/kg/24h)) was calculated and used as a surrogate marker of residual β -cell function at 1, 3, 6 and 12 months after diagnosis. A calculated IDAA1c below 9% was used to define clinical remission. Furthermore, a genetic risk score will be calculated based on the number of risk alleles carried by each child to assess whether the risk score can predict clinical remission within the first year after diagnosis.

Results: A total of 11 candidate genes were significantly regulated by cytokines in human islets and the corresponding risk SNPs analysed in the pediatric cohort. Among the SNPs tested carriers of the risk allele for TNFAIP3 were predicted to have significantly lower C-peptide 12 months after diagnosis ($p=0.02$, adjusted for age, sex and C-peptide at 1 month) as well as significantly higher IDAA1c 9 and 12 months after diagnosis ($p=0.01$ and 0.04 respectively). The expression of TNFAIP3 was significantly upregulated in human islets after cytokine stimulation ($p<0.001$), in line with its protective function in response to cytokines.

Conclusion: The identification of genetic risk variants that affect disease progression will not only increase the knowledge of disease mechanisms involved in the decline of β -cell function. It will also enable the possibility for intervention by enhancing protective effects, such as the effect of TNFAIP3, as well as minimizing deleterious effects of genes and genetic variants.

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Interferon induced with helicase 1 (IFIH1) gene polymorphisms, frequency of human enterovirus infections and islet autoimmunity: longitudinal birth cohort studyE. Witsoe¹, O. Cineke², G. Tapia¹, C.A. Brorsson³, R. Bergholdt⁴, F. Pociot³, L. Stene¹, K. Skjold Ronningen⁵;¹Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway, ²Second faculty of medicine, Charles University Prague, Czech Republic, ³Glostrup Hospital, Denmark, ⁴Hagedorn Research Institute, Gentofte, Denmark, ⁵Oslo University Hospital, Norway.

Background and aims: Interferon induced with helicase C domain 1 (IFIH1) is involved in recognition of and initiation of antiviral activity against enteroviruses. Several polymorphisms of the IFIH1 gene are associated with risk for type 1 diabetes, but it is not established whether these polymorphisms predicts enterovirus frequency in healthy humans. Alleles associated with type 1 diabetes were generally loss of function variants, and were thus hypothesized to predict higher risk of enterovirus infection.

Materials and methods: After screening of 46939 Norwegian newborns, 421 children carrying HLA-DRB1*04:01-DQA1*03-DQB1*03:02/DRB1*03:01-DQA1*05-DQB1*02 genotype (DR4-DQ8/DR3-DQ2, conferring risk for type 1 diabetes) and 375 children without this genotype were included for monthly fecal collections (range 3–35 months). A total of 7793 fecal samples were tested for presence of enterovirus RNA using real time reverse transcriptase PCR.

Results: There was no association of rs1990760, rs35744605, rs35667974, or rs35337543 and enterovirus. For rs35732034 which is predicted to disrupt normal splicing resulting in loss of function, we observed a possible association with frequency of enterovirus of borderline significance: Enterovirus was detected in 26.1% (18/69) of samples from the eight children carrying the A allele, and in 12.4% (955/7724) of samples from the 788 children who were homozygous for the G allele (odds ratio 2.47 (95% CI 1.0–6.3), $p=0.06$. When infections were restricted to those with above median quantity of enterovirus RNA in feces, the odds ratio was 3.27, 95% CI 1.4–7.8, $p=0.01$). Our previously published lack of association between enterovirus frequency and islet autoimmunity was not materially influenced by the IFIH1 SNPs.

Conclusion: We found only partial support for the hypothesis that SNPs in the IFIH1 gene associated type 1 diabetes is a major predictor of enterovirus in healthy young children. This is in contrast to assumptions based on in vitro studies and knock-out mice.

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Family history of autoimmune diseases and genotype and phenotype of children with newly diagnosed type 1 diabetesA. Mäkinen¹, T. Härkönen¹, S. Ryhänen¹, J. Ilonen^{2,3}, M. Knip^{1,4}, the Finnish Pediatric Diabetes Register;¹Scientific Laboratory, Children's Hospital, University of Helsinki and Helsinki University Central Hospital, ²Immunogenetics Laboratory, University of Turku, ³Department of Clinical Microbiology, University of Eastern Finland, Kuopio, ⁴Department of Pediatrics, Tampere University Hospital, Finland.

Background and aims: The aim of this study was to examine the burden of other autoimmune diseases (AID) in the extended families of children with newly diagnosed type 1 diabetes (T1D). We tested the hypothesis that a positive family history of other AID would increase the prevalence of the HLA-DR3-DQ2 haplotype in the children with T1D, and that these children would have a stronger autoimmune reactivity against beta cells reflected by a more frequent and intense autoantibody response (ICA, IAA, GADA, IA-2A).

Materials and methods: We included 1488 children with T1D diagnosed before the age of 15 years (median 8.2, 57% male) from the Finnish Pediatric Diabetes Register. The Register includes over 90% of children annually diagnosed with T1D in our country. Data on clinical markers and the family history of other AID in 1st and 2nd degree relatives at the time of diagnosis were collected with a structured questionnaire, and HLA genotypes and beta cell autoantibodies were analyzed.

Results: Twenty-three children (1.5%) had some other AID already at the time of diagnosis of T1D. The most common diseases were celiac disease ($n=10$) and thyroid dysfunction ($n=9$). These children had ICA less often ($P=0.01$) and had longer duration of symptoms ($P=0.03$), lower beta-OH-butyrate ($P=0.004$) and plasma glucose levels ($P=0.004$), and higher pH

($P=0.001$) at the time of diagnosis compared to the children with T1D only. HLA genetics did not differ between the groups. 31.4% of children had at least one relative with AID other than T1D (thyroid dysfunction: $n=253$, rheumatoid diseases: $n=178$). 11.2% had an affected 1st degree relative and 24.0% had an affected 2nd degree relative. 8.1% had a mother with another AID and 2.8% had an affected father ($P=0.02$). The children with a positive family history of other AID had higher levels of ICA ($P=0.01$) and IA-2A ($P=0.05$). Also the number of children in these families was higher ($P=0.007$). The HLA-DR3-DQ2 haplotype was more common in children with 1st degree relatives affected by other AID ($P=0.01$). Celiac disease in the extended family associated with HLA-DR3-DQ2 haplotype in the index child ($P<0.001$), and rheumatoid diseases with higher levels of ICA ($P=0.003$) and IA-2A ($P=0.007$) and a higher frequency of IAA ($P=0.02$).

Conclusion: Only a small proportion of the index cases had other AID at the time of diagnosis of T1D. One third of the children had a positive family history of AID other than T1D in the extended family. In keeping with the accumulation of many AID among females mothers with other AID were overrepresented in relation to fathers. The HLA-DR3-DQ2 haplotype was associated with a positive family history of other AID, especially celiac disease. There was some support for the hypothesis that children with other AID in the extended family might have stronger beta cell autoimmunity. High ICA titers, in particular, were associated with a positive family history of other AID.

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OP 06 Insulin secretion and exocytosis

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Transcription factor B1 mitochondrial in beta cells and type 2 diabetes

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Background and aims: Recently, we have used information from Genome-wide Association Studies of type 2 diabetes (T2D) and its associated traits, and identified a novel potential culprit in the pathogenesis of the disease: Transcription/translational Factor B1 Mitochondrial (TFB1M). This protein controls mitochondrial protein translation and therefore the expression of mitochondrially encoded genes. We observed that carriers of a common variant of Tfb1m are at increased risk of developing T2D, due to impaired insulin secretion and elevated glucose levels during an oral glucose tolerance test. Perturbed mitochondrial metabolism and impaired insulin secretion were found in Tfb1m^{-/-} mice. To resolve the regulatory role of TFB1M in β -cells and its pathogenetic role in T2D islets, we performed *in vivo* and *in vitro* studies in a murine model of β -cell-specific Tfb1m deficiency (β -Tfb1m^{-/-}).

Materials and methods: To create β -cell Tfb1m^{-/-} mice, RIP-cre transgenic mice were cross-bred with floxed Tfb1m^{+/+} mice. β -cell-specific knock out of TFB1M was assessed by TaqMan qRT-PCR and immunocytochemistry. Plasma glucose concentrations were measured by Infinity Glucose Oxidase Liquid Stable Reagent. Insulin secretion was determined by Ultrasensitive Mercodia ELISA kit.

Results: Quantitative RT-PCR analysis demonstrated a 74% reduction in Tfb1m mRNA expression in pancreatic islets of β -Tfb1m^{-/-} mice compared to controls (floxed Tfb1m^{+/+} mice Cre^{-/-}). Immunocytochemical analysis revealed β -cell specific complete deficiency of TFB1M at the protein level in β -Tfb1m^{-/-} mice. Plasma glucose levels were monitored: at 2.5 month of age β -Tfb1m^{-/-} mice showed retarded elimination of glucose and no increase in insulin secretion during intraperitoneal glucose tolerance test; after 4 months, we found that virtually all β -Tfb1m^{-/-} mice had developed diabetes. Moreover, insulin secretion in response to glucose and α -ketoisocaproic acid is markedly reduced in islets from 2–3 month old β -Tfb1m^{-/-} mice.

Conclusion: Taken together our results obtained with β -Tfb1m^{-/-} mice suggest that Tfb1m deficiency plays a major role in insulin secretion conferring to this protein, identified as a genetically associated T2D risk factor, a specific biological role in the pathogenesis of the disease. We suggest that the final effect on insulin secretion due to Tfb1m deficiency may underline how perturbation in the mitochondrial proteome homeostasis is a condition which finally leads to the development of T2D. Further work will help in the validation of potentially new therapeutic targets.

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Reduced expression of genes involved in exocytosis in type 2 diabetic human islets

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Background and aims: Recent studies suggest that impaired insulin secretion occurs already before the onset of type 2 diabetes (T2D). This makes processes involved in the exocytosis of insulin critical during the development of the disease. Exocytosis precedes insulin secretion by means of targeting the insulin-containing granules to the plasma membrane and mechanical fusion of the granular membranes to the cell plasma membrane. Successful β -cell exocytosis involves gene expression of several proteins such as SNAP-25, STX1A, VAMP2 and STXBP. We hypothesise that expression of genes involved in the exocytotic process is altered in T2D individuals, and that changes in gene expression would correlate to changes in HbA1c and GSIS (glucose stimulated insulin secretion).

Material and methods: Human pancreatic islets from 55 non-T2D and 9 T2D deceased donors in Scandinavia were obtained and processed onto a microarray chip measuring the expression of 21 000 genes where 1300 was found to be down-regulated in T2D subjects. We then compared the mRNA expression of 23 exocytotic genes on the microarray between T2D human

donors and non-T2D subjects using Mann-Whitney U-test. The resulting number of nominally down-regulated genes was surveyed by a Chi-2 test where $p < 0.05$ was considered adequate to determine if a significant difference exists between the expected frequencies and the observed frequencies. Where the expression pattern differed technically, three genes were replicated using quantitative RT-PCR. The gene expression was further correlated to HbA1c *in vivo* and measurements of GSIS *in vitro* from the same donors, using Spearman correlation coefficient.

Results: In the microarray, 5 out of the 23 genes were nominally down regulated ($p < 0.05$) e.g. STX1A expression was reduced by 26 % ($p = 0.010$) in T2D individuals as compared to the control group. The Chi-2 test established that the number of down-regulations in our data set was higher than could be expected ($p < 0.05$) from the total number of down-regulations on the microarray. Three of these genes, one being STX1A, were additionally selected for replication in qPCR which strengthened the trend seen in the microarray such as STX1A expression being reduced by 40% ($p = 0.027$) in human islets from T2D donors compared to healthy individuals. Further, the gene expression of all five genes correlated negatively to HbA1c ($p < 0.04$) where STX1A had a p -value of 0.005, and positively to GSIS ($p < 0.05$) where STX1A had a p -value of 0.004. None of the genes were associated to basal insulin secretion. **Conclusion:** Our study implies that mRNA expression of genes important for exocytosis is down-regulated in T2D individuals. Further, we can correlate these genes to HbA_{1c} and GSIS. In combination, these findings show that decreased expression of exocytotic genes may contribute to the deregulated glucose stimulated insulin secretion in T2D.

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Nor-1 is a novel incretin-sensitive regulator of the rat insulin genes

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Background and aims: The nuclear receptor subfamily NR4A (nuclear receptor subfamily 4, group A), comprising Nur-77 (NR4A1), Nur-1 (NR4A2), and Nor-1 (NR4A3), is a highly conserved group of ligand-independent transcription factors and early-response genes. They are rapidly regulated at the transcriptional level by various stimuli and modulate differentiation, proliferation, apoptosis, and lipid and glucose metabolism. We could recently show that common genetic variation within the NR4A3, but not within the NR4A1 or NR4A2, locus affects insulin secretion in humans providing evidence for an important role of Nor-1 in human β -cell function. In keeping with this, we detected relevant NR4A3 expression levels in human islets. In this study, we aimed to investigate Nor-1's molecular function in pancreatic β -cells.

Materials and methods: We used RNA interference-mediated gene knock-down, chromatin immunoprecipitation, and pharmacological activators and inhibitors to study the molecular role of Nor-1 in the rat insulinoma cell line INS-1E.

Results: Nr4a3 knock-down significantly reduced Ins1 mRNA expression ($p < 0.05$) and tended to reduce Ins2 mRNA expression ($p = 0.07$, $n = 4$). In addition, Nr4a3 knock-down resulted in reduced glucose-stimulated insulin secretion ($p < 0.05$). Conversely, incubation of INS-1E cells with the Nor-1 activator 6-mercaptopurine significantly induced Ins1 and Ins2 mRNA expression ($p < 0.05$ both). Furthermore, we were able to demonstrate Nor-1 binding to a specific binding site in the Ins1 promoter. Having shown Nr4a3 involvement in insulin expression and secretion, we asked how Nr4a3 expression is regulated in INS-1E cells. Since Nr4a3 is known to be induced in the liver via cAMP/PKA, a pathway activated by incretins in the pancreatic β -cell, we analysed Nr4a3 expression after incubation with exendin-4. The Nr4a3 mRNA content significantly increased upon exendin-4 treatment, and this increase was even larger in cells co-incubated with high glucose concentrations ($p < 0.05$ all). The adenylate cyclase activator forskolin and dibutyryl-cAMP significantly induced the mRNA expression of Nr4a3, while the PKA inhibitor H89 reduced its expression ($p < 0.05$ all). Furthermore, Creb1 knock-down abolished the exendin-4/high glucose-mediated Nr4a3 induction.

Conclusion: Nor-1 represents a novel transcriptional regulator of the rat insulin genes which can be induced by incretins plus high glucose via the cAMP/PKA/Creb-1 pathway. Since Nor-1 binding sites were also identified in the human insulin gene, the proposed role of Nor-1 in insulin expression/secretion may also hold for human β -cells.

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Deletion of the Cited2 gene in beta cells causes impaired insulin secretion and glucose intolerance in miceJ. Cantley¹, A. Davenport¹, S.L. Dunwoodie², T.J. Biden¹;¹Diabetes and Obesity Program, Garvan Institute of Medical Research,²Developmental Biology Division, Victor Chang Cardiac Research Institute, Sydney, Australia.

Background and aims: Beta cell failure is a hall mark of type2 diabetes (T2D). Two of the key questions in the aetiology of T2D are: What are the molecular mechanisms controlling normal beta cell function? And how do these fail during T2D? We have recently implicated the gene Cbp/p300-interacting transactivator 2, with Glu/Asp-rich carboxy-terminal domain, 2 (Cited2) as being potentially involved in controlling beta cell function. Cited2 has been reported to act as a transcriptional cofactor in a number of different cell types, and to regulate the activity of a number of transcription factors, some of which are known to play an important role in beta cell function. Despite Cited2 being highly expressed, its role in beta cell function has not been investigated. Therefore, the aim of our study was to investigate the role of Cited2 in beta cells.

Materials and methods: We have deleted Cited2 in the beta cells of mice (β Cited2KO) by crossing Cited2 floxed mice with Ins2cre mice expressing cre-recombinase under the control of the insulin2 promoter. We assessed glucose homeostasis and islet morphology. We also measured Cited2 expression in models of beta cell dysfunction by RT-PCR or gene array.

Results: β Cited2KO mice were indistinguishable from control mice (Ins2cre and Cited2floxed) in terms of body mass, insulin action and appearance. However, when we performed an i.p. glucose tolerance test (GTT) at 12 weeks of age, we found that both male and female β Cited2KO mice were significantly glucose intolerant relative to controls (Male GTT AUC mean \pm SEM: Control 1547 \pm 58.4 n=16; β Cited2KO 2367 \pm 78.7 n=8; $P<0.001$ by ANOVA with bonferroni post-test). β Cited2KO mice were found to have reduced insulin levels during the glucose tolerance test relative to control mice, which indicated that beta cell Cited2 deletion was disrupting normal beta cell function. Morphometric histological analysis revealed normal beta cell mass (mg mean \pm SEM: Control 1.562 \pm 0.275; β Cited2KO 1.856 \pm 0.232mg; n=3, NS by t-test) but altered islet architecture, suggesting that Cited2 may regulate insulin secretion and islet organisation, rather than beta cell mass. Further indication of the important role Cited2 plays in beta cells came from observations that the Cited2 transcript was down regulated by 50% in Min6 cells treated with the saturated fatty acid palmitate, a model of lipotoxicity and secretory dysfunction, using gene expression arrays (n=2). We also studied Db/Db mice which are a model of beta cell dysfunction and type2 diabetes, and found a 38% downregulation of Cited2 mRNA in isolated Db/Db islets relative to controls (n=12, $P<0.01$ by t-test). Moreover, Cited2 expression was unaltered in islets isolated from Ob/Ob mice which show obesity but have preserved beta cell function and do not develop diabetes.

Conclusion: Our data clearly show that Cited2 is required for normal beta cell function, and that reduced Cited2 expression levels are associated with beta cell dysfunction in models of diabetes. Although more work is needed to fully explore the role of Cited2 in the beta cell, we propose that Cited2 is a critical regulator of gene expression in beta cells, exerting control over beta cell function, insulin secretion and glucose homeostasis.

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The Ser473 AKT specific phosphatase PHLPP plays a key role in beta cell dysfunction

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Background and aims: Chronic exposure of pancreatic beta-cells to high glucose levels is associated with reduced insulin secretion and synthesis, and decreased cell survival as well as with the impairment of several steps along the insulin signaling pathway, including a reduced phosphorylation of the kinase Akt on the Serine 473 (Ser473), but not on the Threonine 308 (Thr308) residue. We thus hypothesized that the Ser473 specific phosphatase PHLPP1/2 may have a role in glucose-induced beta-cell dysfunction. In addition, since it has been reported that glucagon-like peptide 1 (GLP-1) and its analogues improve beta-cell homeostasis promoting Akt phosphorylation, we wished to test the hypothesis that down-regulation of PHLPP1/2 may be an additional mechanism underlying GLP-1 action.

Materials and methods: INS-1 rat beta-cells were cultured at physiological (11.1 mmol/L, INS-1 LG) or high (30 mmol/L, INS-1 HG) glucose concentrations for 10-15 passages. To evaluate GLP-1 ability to reduce PHLPP1/2 expression, time-course and dose-response experiments were performed treating INS-1 HG with 0.3, 3 and 30 mmol/L GLP-1 for 24, 48, 72, 96 hrs, the treatment was carried out in the presence of 20nmol/L dipeptidyl peptidase-IV inhibitor to prevent GLP-1 degradation. Real-Time RT-PCR was used to assess mRNA levels and Western blots were performed to evaluate protein expression and the activation state of Akt and its substrates. Statistical differences were assessed by Student's t test or Two-Way Anova, a p value of 0.05 was considered statistically significant.

Results: We confirmed that, in our cellular model, chronic exposure to high glucose significantly impairs glucose-stimulated insulin secretion ($p<0.0001$, n= 3), insulin synthesis ($p<0.0001$, n=6) and cell survival, assessed using the expression levels of the pro-apoptotic protein Bad, which showed a 1.75-fold increase, and of the anti-apoptotic protein BclxL, which by contrast was decreased by 62%, as a read-out for apoptosis ($p<0.05$, n=3, for both markers). Notably, in INS-1 HG the expression of PHLPP1/2 was significantly increased both at mRNA (PHLPP1 250 \pm 22%; PHLPP2 320 \pm 24%, $p<0.05$, n=10) and protein (PHLPP1 132 \pm 22%; PHLPP2 176 \pm 34%, $p<0.05$, n=4) level. This increase was paralleled by a significant decrease of Akt phosphorylation on Ser473 after stimulation with insulin 100 nmol/L for 10 mins (-50 \pm 12%, $p<0.05$, n=6); by contrast the phosphorylation of Thr308 was only slightly, and not significantly, reduced. The decreased activation of Akt was mirrored by a decreased insulin-stimulated phosphorylation of the major Akt substrates: -44.4 \pm 9% for p70S6K ($p=0.0011$, n=6), -57.5 \pm 2.12% for pElF2alpha ($p=0.013$, n=3), -48 \pm 2.82% for mTOR ($p=0.0026$, n=3), and -58.67 \pm 11.2% for Foxo-1 ($p=0.0009$, n=3) in INS-1 HG. These results suggest that PHLPP1/2 may indeed play an important role in glucose-induced beta-cell dysfunction, we therefore proceeded to evaluate if GLP-1 ability to counteract the effects of hyperglycemia could be mediated by the down-regulation of PHLPP1/2 expression; indeed our results show that GLP-1 treatment significantly reduces PHLPP1/2 mRNA levels in INS-1 HG cells, with a maximal effect for the 0.3 mmol/L GLP-1 dose at 24h (PHLPP1 -40 \pm 19%; PHLPP2 -33 \pm 12%, $p<0.01$, n=8).

Conclusion: Our results, contributing to the elucidation of the mechanisms by which hyperglycemia affects the multiple functions controlled by Akt, could provide indications for novel therapeutic approach to deal with beta-cell failure.

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Beta-arrestin 2 is required for insulin signalling in pancreatic beta-cells

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Background and aims: The autocrine action of insulin on its own receptor was shown to be critical for the maintenance of normal pancreatic β -cell function and mass. Recently, it has been reported that in the downstream signalling of the insulin receptor (IR), besides the conventional IRS/PI3K/Akt pathway, a second pathway involving β -arrestin 2 (β -arr2) is required to induce a full Akt activation in hepatocytes. So, β -arr2 was shown to scaffold a complex with Akt, Src and IR in response to insulin and any deficiency of this signal contributed to the development of insulin resistance. The aim of our study was to investigate the implication of the β -arr2/Src signalling complex in insulin-mediated Akt activation in pancreatic β -cells.

Materials and methods: To examine the mechanism(s) by which insulin activates Akt, we assessed the Akt phosphorylation status by Western blotting with phospho-specific antibodies that recognize phosphorylated Akt on Ser473 or Thr308. Free cytosolic [Ca²⁺] changes from mouse islets were recorded using Fura2-AM and insulin secretion was measured using perifused islets.

Results: In INS-1E cells, insulin dose-dependently (1-100nM) induced a rapid Akt phosphorylation on both Thr308 and Ser473, with a maximum (~3 to 4-fold increase) reached after 10 min of stimulation. Interestingly, while wortmannin, a PI3K inhibitor, completely abolished the Akt phosphorylation, PP2, a selective inhibitor of the tyrosine kinase Src, reduced the Akt phosphorylation on both Ser473 and Thr308 by 40% and 20%, respectively. Besides, β -arr2 depletion using short interfering RNA reduced by 40% the Akt phosphorylation. These results suggest that not only PI3K but also Src and β -arr2 are required for insulin-induced full Akt activation. In addition, we showed by immunoprecipitation that insulin induced an association between Akt and

the IR as well as β -arr2 and Src at 5 min for at least 30 min in INS-1E cells. Finally, the biological relevance of both Src and β -arr2 involvements were confirmed in mouse pancreatic islets. Indeed, inhibition of Src (with PP2) as well as knockdown of β -arr2 (β -arr2 KO mice) decreased by 40–80%; respectively, the insulin-induced Akt activation. Moreover, insulin-induced phosphorylations of the transcription factor FoxO1 and the glycogen synthase kinase 3, two downstream targets of Akt, were reduced in β -arr2 KO mouse islets. At last, since we observed that insulin can recruit β -arr2 through its receptor, and that insulin was reported to regulate its own secretion, we have assessed the role of β -arr2 in β -cell function using β -arr2 KO mice. Female β -arr2 KO mice exhibited slight glucose intolerance and lower fed plasma insulin level. Nevertheless, glucose-induced free cytosolic $[Ca^{2+}]$ changes and insulin secretion from perfused islets were not affected. However, the insulin content of both the whole pancreas and isolated islets were significantly decreased in β -arr2 KO mice (~25%), suggesting a role of β -arr2 in the modulation of β -cell mass.

Conclusion: We report a new process in pancreatic β -cells by which insulin recruits β -arr2 to scaffold Src to IR for full Akt activation and thus provide new insights concerning the mechanisms of insulin action in pancreatic β -cells.

OP 07 Nephropathy: epidemiology and clinical trials

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Chronic kidney disease and mortality risk among elderly diabetic individuals (ZODIAC-24)

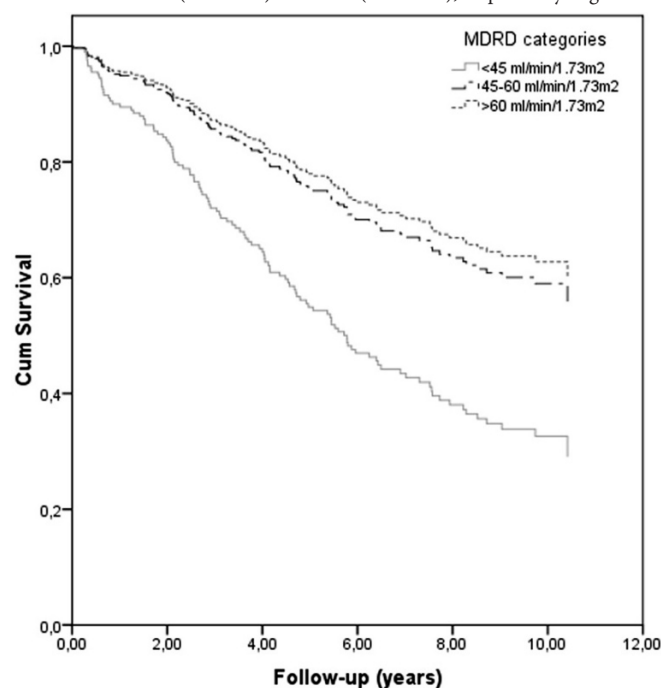
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Background and aims: A decreased renal function and albuminuria are associated with increased mortality in patients with type 2 diabetes mellitus (T2DM). Since it is unclear whether these associations remain similar in elderly it was our aim to study these associations in elderly patients with T2DM.

Materials and methods: Between 1998 and 1999, 1022 primary care patients ≥ 60 years with T2DM participated in the ZODIAC study, a prospective observational study. Data on mortality were collected in 2009. A Cox proportional hazard model was used to investigate the association of the modification of diet in renal disease formula (MDRD), the Cockcroft-Gault formula (CG) and albuminuria with mortality. Analyses were performed in strata according to age: 60–75 ($n=648$) and >75 ($n=374$) years. Age, gender, smoking (dichotomous), body mass index, systolic blood pressure, diabetes duration, macro vascular complications (dichotomous), total cholesterol-HDL ratio, and HbA1c were selected as possible confounders. Three different models were used. Model 1 was the crude model, model 2 included all selected confounders, model 3 used all selected confounders except for those variables which were already used in the eGFR prediction equations itself.

Results: After a median follow-up time of 10 years, 537 of 1022 patients died (42% was due to cardiovascular causes). In patients aged 60–75 years, a MDRD <45 and 45–60 ml/min/1.73m² were associated with increased cardiovascular mortality: HR 3.05 (95%CI 1.44–6.47) and HR 1.69 (95%CI 1.02–2.79), respectively; for CG hazard ratios were HR 6.21 (95%CI 2.76–13.95) and HR 2.33 (95%CI 1.35–4.05), respectively. In patients >75 years increased cardiovascular mortality was only observed when CG or MDRD were <45 ml/min/(1.73m²); HRs 1.93 (95%CI 1.93–3.64) and 2.27 (95%CI 1.37–3.78), respectively. Compared to participants aged 60–75 years with a MDRD >60 ml/min/1.73m² without albuminuria, the fully adjusted HRs for cardiovascular mortality were 2.59 (95%CI 1.23–5.45) for impaired MDRD (45–60 ml/min/1.73m²) without albuminuria, and 3.91 (95%CI 1.91–8.02) for those with a MDRD of 45–60 ml/min/1.73m² with albuminuria. For participants aged >75 years, the HRs were 1.23 (0.61–2.42) and 2.12 (1.06–4.25), respectively. Figure 1 il



illustrates the increased cardiovascular mortality rates for patients >75 years with an eGFR <45 ml/min/1.73m².

Conclusion: Patients >75 years with a MDRD or a CG of 45–60 ml/min/(1.73m²) are not at increased risk for all-cause or cardiovascular mortality. Albuminuria on the other hand, is strongly associated with increased all-cause and cardiovascular mortality at all levels of eGFR, also in elderly patients.

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Association of normoalbuminuric renal impairment with cardiovascular disease

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Background and aims: In type 2 diabetes, prevalence of nonalbuminuric renal impairment, as identified by an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m² without albuminuria, is increasing worldwide, though the prognostic implications of this phenotype in terms of both cardiovascular disease (CVD) risk and renal outcome are poorly defined. This study was aimed at evaluating the reciprocal association of nonalbuminuric renal impairment with CVD risk factors and prevalent major acute CVD events, in patients with type 2 diabetes.

Materials and methods: We used baseline data from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study. The RIACE cohort consists of 15,773 patients with type 2 diabetes, consecutively visiting 19 Diabetes Clinics in years 2007–2008. Exclusion criteria were dialysis or renal transplantation. GFR was estimated from serum creatinine using the simplified Modification of Diet in Renal Disease equation, albuminuria was measured by immunonephelometry or immunoturbidimetry. Chronic kidney disease (CKD) was defined as an eGFR <60 ml/min/1.73 m² and/or micro/macroalbuminuria. Previous major acute CVD events were recorded based on hospital discharge records.

Results: No CKD was detected in 9,865 patients (62.6%), CKD stages 1–2 (i.e. eGFR ≥60 ml/min/1.73 m² and albuminuria) in 2,949 (18.7%), and CKD stages 3–5 (i.e. eGFR <60 ml/min/1.73 m² with or without albuminuria) in 2,959 (18.8%). Among subjects with renal impairment, 56.6% were nonalbuminuric and 43.4% were albuminuric (30.8% microalbuminuric and 12.6% macroalbuminuric). At least one major acute CVD event was adjudicated in 3,654 patients, who were older, more frequently male and on insulin treatment, and with longer diabetes duration, higher HbA_{1c}, triglycerides and prevalence of albuminuria, CKD, and retinopathy, and lower HDL-cholesterol and eGFR than those without events. Reduced eGFR without albuminuria was more strongly associated with CVD events than normal eGFR with micro or macroalbuminuria (OR 1.52, 95%CI 1.34–1.73 vs. 1.20, 1.08–1.33), but less strongly than reduced eGFR with albuminuria (1.90, 1.66–2.19). Interestingly, nonalbuminuric renal impairment was associated with coronary events (myocardial infarction and/or coronary revascularization) more strongly than albuminuric renal impairment and albuminuria with normal eGFR, whereas the association of the nonalbuminuric phenotype with cerebro-vascular (stroke and/or carotid revascularization) and peripheral (ulcer/gangrene/amputation and/or lower limb revascularization) events was lower than for the other two forms of CKD.

Conclusion: This large-cohort study shows that nonalbuminuric renal impairment in type 2 diabetes, though encumbered by lower cardiovascular risk and prevalence of CVD than the albuminuric phenotype, is strongly associated with coronary events.

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Intensive glucose lowering and end stage kidney disease: new data from the ADVANCE trial

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Background and aims: Blood glucose levels have been linked to the risk of kidney disease, but the effects of intensive glucose control on major kidney outcomes among people with diabetes are not known.

Materials and methods: This analysis from the ADVANCE trial compared the effects of an intensive glucose lowering target (HbA_{1c} < 6.5%) using a glizalide MR based regimen to a standard target (HbA_{1c} < 7%) on major renal events. The outcomes assessed were end-stage kidney disease (ESKD, defined as maintenance dialysis or transplantation), renal death, confirmed doubling of creatinine to above 200 µmol/L, and sustained doubling of creatinine to the same level (final recorded value consistent with the doubling criteria).

Results: 11,140 patients were randomised to intensive or standard glucose lowering. The mean achieved HbA_{1c} levels were 6.5% in the intensive arm, and 7.3% in the standard arm. After an average follow up of 5 years, the risk of ESKD was significantly lower in the intensive compared to the standard glucose lowering arm: hazard ratio [HR] 0.35, 95% confidence interval [CI] 0.15–0.83, p = 0.02. There was a non-significant trend towards benefit for renal death (HR 0.85, CI 0.45–1.63, p = 0.63) and the composite of ESKD and renal death (HR 0.64, CI 0.38–1.08, p = 0.09). No clear effect on doubling of creatinine (HR 1.15, CI 0.82–1.63, p = 0.42) or sustained doubling of creatinine 0.83 (0.54–1.27, p = 0.39) was observed.

Conclusions: An intensive blood glucose lowering regimen based on glizalide MR reduced the risk of ESKD in the ADVANCE study, but the effects on other renal outcomes were less clear. The interpretation of doubling of creatinine as a component of renal endpoints requires further consideration. Additional studies of glucose lowering in people with diabetes at high risk of ESKD are needed.

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Microvascular effects of intensive blood pressure control and its relation to glycaemic control in the ACCORD blood pressure trial

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Background and aims: Reduction of blood pressure (BP) and blood glucose diminish some microvascular complications of type 2 diabetes, but data on the combined effects of these interventions are sparse. Here we report effects of intensive BP control alone and in combination with intensive glycemic control on microvascular complications in the ACCORD BP trial.

Materials and methods: 4733 adults with type 2 diabetes (T2DM) and systolic BP (SBP) 130–180 mm Hg on 0–3 BP medications were randomized to intensive (target SBP <120 mm Hg) or standard (SBP <140 mm Hg) BP control, and separately randomized to intensive (target HbA_{1c} <6.0%) or standard (HbA_{1c} 7.0–7.9%) glycemic control. Pre-specified outcomes included one composite microvascular outcome measure (dialysis or renal transplantation, high serum creatinine [>3.3 mg/dL], or retinal photocoagulation or vitrectomy) and 9 single measures of kidney, eye, or peripheral nerve function. Proportional hazards regression models were used to assess two-way interactions

between glycemia and BP treatment arm assignment for each microvascular complication.

Results: Over a mean follow-up of 4.7 years, the primary microvascular outcome occurred in 527 of 4733 participants, including 11.4% in the intensive BP arm and 10.9% in the standard BP arm (HR=1.08, 95% CI: 0.91–1.28). Whereas intensive glycemic control reduced the incidence of macroalbuminuria and a few other microvascular outcomes, intensive BP control only reduced development of microalbuminuria (HR=0.84, 95% CI:0.72–0.97). The observed reductions in microvascular outcomes by intensive glycemic control were not affected by the BP treatment arm (no interaction).

Conclusion: Intensive BP control improved only 1 of 10 pre-specified microvascular outcomes. None of the pre-specified outcomes were further significantly reduced in participants intensively treated for both glycemia and BP compared to those treated with either regimen alone, signifying the lack of an additional beneficial effect from combined intensive treatment.

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Association of renal status and cardiovascular risk, and safety of fenofibrate in renal impairment in the FIELD study of 9,795 subjects with type 2 diabetes

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Background and aims: Renal function predicts cardiovascular disease in Type 2 diabetes. Fenofibrate treatment raises plasma creatinine. In the Fenofibrate and Event Lowering in Diabetes (FIELD) study, we examined the effect of renal status changes on CVD risk and the consequences of fenofibrate administration in subjects with moderate renal impairment, where safety of treatment has been uncertain.

Materials and methods: In FIELD, 9795 patients, 50–75 years with type 2 diabetes, were randomly assigned to co-micronised fenofibrate 200mg daily or matching placebo over 5 years. The pre-specified outcome for subgroup analysis was total cardiovascular events. Renal status (eGFR [MDRD-4 variable] and albuminuria [albumin:creatinine ratio]) was examined (baseline, year 2 and close-out). End-stage renal events were recorded. Analysis was by intention-to-treat.

Results: Higher CVD risk was strongly associated with lower baseline eGFR (HR for eGFR<60: 1.44 [95%CI 1.2–1.8, p<0.001]) and with albuminuria (HR 1.3 [95%CI 1.2–1.5], p<0.001); and was modified by renal status changes over the first 2 years. In those on placebo whose albuminuria status changed from normal to abnormal, risk significantly increased (by about 30%, p=0.006) whereas it reduced when albuminuria resolved (by about 25%, p=0.01). A similar pattern was observed for changing eGFR status between < 60 and ≥ 60 ml/min/1.73m². Overall, fenofibrate reduced total cardiovascular events by 11% (p=0.035), with comparable benefits seen in all eGFR subgroups (p-interaction=0.2). The greatest absolute CVD risk reduction (7.5%) with fenofibrate was observed in those with moderate renal impairment, eGFR 30–59 ml/min/1.73m² (HR 0.68, p=0.035), without any safety concerns. Lesser loss of renal function (1.0 ml/min/ 1.73m² less per annum) occurred over 5 years among those allocated fenofibrate than placebo (p=0.0003; wash-out study, n=661). End-stage renal disease events were no more common with fenofibrate, including among those with moderate renal impairment (see table), or those with the greatest early creatinine rise on fenofibrate.

Conclusion: Lower eGFR and albuminuria are both strongly independently associated with CVD risk. Improvements over time are associated with lower CVD risk. Fenofibrate slows eGFR loss and does not cause renal injury in diabetes or reduce cardiovascular benefits when creatinine rises. There were large cardiovascular benefits and no renal issues when used in those with moderate (eGFR 30–59 ml/min/1.73m²) renal impairment.

End-Stage Renal Disease Events by Baseline Renal Function and Treatment

Treatment allocation (n) Pbo=Placebo; Feno=Fenofibrate	eGFR 30–60		eGFR [60–90]		eGFR ≥ 90		Total	
	Pbo (224)	Feno (295)	Pbo (2657)	Feno (2561)	Pbo (2019)	Feno (2039)	Pbo (4900)	Feno (4895)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
End-stage renal disease events*	5 (2.2)	7 (2.4)	14 (0.5)	10 (0.4)	7 (0.3)	4 (0.2)	26 (0.5)	21 (0.4)

* creatinine > 400μmol/L, dialysis, renal transplant or death

Clinical Trial Registration Number: ISRCTN64783481

Supported by: Abbott Laboratories

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Simultaneous kidney and pancreas transplantation, compared to hyperglycaemia, improves long-term (>8 yrs) outcome in the transplanted kidney

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Background and aims: To assess the impact of long-term glycemic control on renal transplant function and structure, we compared type 1 diabetes patients who had either received combined kidney-pancreas grafts from deceased donors, or a single kidney graft from live donors (LD), more than 8 years ago.

Materials and methods: Eighteen type 1 diabetes patients transplanted with simultaneous pancreas and kidney grafts (SPK) were compared to 16 patients transplanted with single kidneys (SK) from LD. These two categories of patients tend to be comparable in terms of age and comorbidity at transplantation, and the patients were randomly selected among living subjects. The immunosuppressive regimen was CNi-based and essentially the same in both groups. Estimated GFR (eGFR) was calculated in a stable phase 6 months after transplantation and then after 8–15 years. At follow-up a kidney graft biopsy was also obtained, and measurements of glomerular basement membranes by interactive computerized image analysis of electron micrographs (EM) were performed.

Results: Age at baseline was similar (mean (SEM)): SPK: 40.3 (1.3) SK: 39.3 (2.5) years. Baseline BMI and eGFR were also similar: 23.0 (0.6) kg/m² vs 23.9 (0.8), and 51.3 (3.2) vs. 53.0 (4.0) ml/min/1.73 m², respectively. The SPK group was followed for 13.6 (1.1) years, and the SK group 10.8 (0.6) years. HbA1c at follow-up was 5.7 (0.2) % and 8.3 (0.5) % in the SPK and SK groups, respectively (p<0.01). At follow up, eGFR had increased 4.8 (5.9) ml/min/1.73 m² in the SPK group, and fallen 10.3 (4.2) ml/min/1.73 m² in the SK group (p=0.03). EM showed a 30% thicker basement membrane in the SK patients: SPK: 295 (17.1) nm, SK: 380 (31.3) nm (p=0.08). Serum metalloproteinase-9 (MMP-9) was lower in the SPK patients compared to the SK patients; MMP-9: 37.3 (8.9) vs 56.3 (11.4) ng/ml, while its inhibitor TIMP-1 was the same in each group.

Conclusion: Long-term GFR is better preserved in kidney transplanted type 1 diabetes patients when glucose homeostasis is maintained with a functional pancreas graft. The glomerular basement membrane apparently remains thinner in these patients.

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OP 08 Stem cells and regeneration

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Gestational glucose intolerance resulting from impaired beta cell mass expansion in the absence of survivin in female mice

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Background and aims: Adaptive beta cell mass expansion ensures metabolic balance in pregnancy, a physiological state marked by increased insulin demand due to the insulin resistance. Beta cell proliferation is the principal mechanism of beta cell mass expansion in pregnancy, but the molecular mechanisms responsible for this process is elusive. Our previous data showed that survivin regulates beta cell mass and beta cell proliferation during perinatal period and after duct ligation. We predicted that decreased survivin activity could result in an inability to expand beta cell mass during times of increased metabolic demand. This study was designed to determine whether survivin was required for glucose homeostasis and beta cell mass expansion in maternal islets during pregnancy.

Materials and methods: Using the Cre-loxP recombination system, we generated a rat insulin promoter (RIP)-driven survivin knockout mouse with a specific deletion of survivin in pancreatic beta cells. Survivin expression profile, beta cell mass, beta cell proliferation and glucose homeostasis were assessed in adult female RIPCre⁺survivin^{lox/lox} mice and their control littermates (RIPCre⁺survivin^{+/+}) during pregnancy.

Results: In pregnant control mice, maternal beta cell mass increased by two-fold and beta cell replication peaked at gestational day(GD)14.5. Transient re-expression of survivin was detected in the islets from GD10.5 to GD18.5 with the peak at GD14.5, which closely mirrored the beta cell proliferative profile during pregnancy. Targeted deletion of survivin in beta cells, female mutant mice maintained glucose homeostasis at eight weeks, despite a 50% reduction in beta cell mass due to the lack of survivin during perinatal period. However, pregnant female mutant mice at GD14.5 exhibited markedly higher glucose excursions during an IPGTT and compromised insulin secretion after i.p. glucose injection compared with controls, with specific impairment in beta cell mass expansion and the failure of the increase of beta cell proliferation.

Conclusion: Our results indicate that transient reexpression of survivin in the pancreatic beta cells during pregnancy is essential for beta cell mass expansion. Survivin deficiency in beta cells leads to defects in beta-cell proliferation, resulting in a decrease in beta cell mass expansion and glucose intolerance during pregnancy.

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PI3K-C2α regulates proliferation and differentiation of pancreatic beta cells

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Background and aims: PI3K-C2α generates PI(3,4)P₂ in beta cells and is involved in glucose-stimulated insulin release via a mechanism involving PKBa activation, TBC1D4 phosphorylation and regulation of glucokinase expression. We have recently shown that knock-down of PI3K-C2α in pancreatic beta cells, in contrast to most other cells, leads to increased proliferation and survival. The aim of this study was to evaluate the mechanism underlying the increased beta cell proliferation and survival after knock-down of PI3K-C2α.

Materials and methods: MIN6 cells, primary mouse and human beta cells were treated with control siRNA or siRNA against PI3K-C2α. Knock-down of protein levels of PI3K-C2α was verified by Western blotting (80% knock-down). Cell proliferation under different conditions was measured by BrdU- or EdU- incorporation. Protein expression and phosphorylation were evaluated by Western blot. PKBa activity was measured by Akt1/PKBa immunoprecipitation kinase assay. Apoptosis rate of MIN6 cells after treatment with either H₂O₂ or staurosporine was determined by triple staining with Hoechst 33342, propidium iodide (PI) and AlexaFluor488-annexinV, where AlexaFluor488-annexinV positive PI-negative stained cells were considered apoptotic.

Results: The area covered by BrdU-positive nuclei related to the area covered by all cells was increased 2.3 ± 0.12fold in PI3K-C2α siRNA-treated MIN6 cells compared to control siRNA-treated MIN6 cells. BrdU- and EdU-incorporation were also increased in mouse (1.88 ± 0.24fold) and human (1.92 ± 0.21fold) primary beta cells treated with PI3K-C2α siRNA compared to control siRNA-treated cells. Cells treated with PI3K-C2α siRNA were more resistant to apoptotic stimuli (48.4 ± 4.9% less apoptotic cells when treated with 20 μM H₂O₂ and 32 ± 2.9% less apoptotic cells when treated with 6 μM staurosporine). Knock-down of PI3K-C2α in MIN6 cells affected also cell size (cells were 37.9 ± 2.6% smaller), glucokinase protein expression (25 ± 3% less), first phase of glucose-stimulated insulin release (-25 ± 5.9 %) and abolished the insulin-stimulated activation of PKBa and downstream PKB target proteins (FoxO1, TCS2, TBC1D4). Basal PKBa activity did not change. The positive effect of PI3K-C2α knock-down on MIN6 cell proliferation was abolished by blocking PI3K class Ia (20 μM LY294002), by IR-blocking antibodies and by inhibiting PKBa (0.1 μM Akti-1/2). Knock-down of PKBa in MIN6 cells led to decreased proliferation (-33.9 ± 5.3%) and increased apoptosis, the latter both under normal culture conditions (+100 ± 9.9%) and when challenged with apoptotic stimuli H₂O₂ or staurosporine (+50 ± 5.2%).

Conclusion: PI3K-C2α and PKBa both contribute to the regulation of beta cell proliferation and differentiation. PI3K-C2α is a negative regulator of beta cell proliferation and maintains beta cell differentiation and function via a pathway involving signaling through insulin receptors, PI3K class Ia and PKBa.

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MicroRNA profiling during expansion and differentiation of human islet-derived precursor cells reveals stage specific microRNA signature

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Background and aims: Generation of insulin producing cells from stem/progenitor cells is potentially crucial to develop a proper cell therapy for type 1 diabetes. MicroRNAs (miRs) are small endogenous RNAs representing important negative regulators of gene expression. We have previously obtained human pancreatic islet derived mesenchymal (hPIDM) cells that can be *in vitro* expanded and induced to re-acquire a pancreatic endocrine phenotype when cultured in appropriate conditions. In order to gain insights into the mechanisms regulating gene expression during beta cell regeneration and differentiation, we here performed an extended miR profiling in hPIDM cells during expansion and their differentiation into insulin producing cells in comparison with human native pancreatic islets.

Materials and methods: hPIDM cells, obtained from human pancreatic islets purified from 3 organ donors, were expanded in medium containing 10% FBS. For differentiation, hPIDM cells were cultured for 21 days in serum-free medium containing insulin, transferrin and Na-selenite. Real time PCR was used for gene expression analysis of 92 genes (associated to a mesenchymal or to a pancreatic endocrine phenotype). MiR profiling (762 human miRs) was performed using TaqMan array cards. Genes and miRs expression were evaluated at the following stages: primary human islets, hPIDM cells during expansion and after differentiation. In addition, for miRs of interest, a six points time course expression analysis was performed during the 21 days of differentiation.

Results: Gene expression analysis confirmed that, during expansion, hPIDM cells acquired mesenchymal-associated genes (e.g. vimentin, nestin) and lost islet-related genes (e.g. insulin, glucagon, somatostatin, etc.). Upon differentiation, hPIDM cells re-acquired islet-associated genes with a major reduction of mesenchymal-associated ones. MiR profiling showed a stage specific differential expression of several miRs, including some involved in stemness regulation or islet function and development. Specifically miR-302-367 cluster, involved in self-renewal and in stemness maintenance, were absent in native islets and appeared in proliferating hPIDM cells, which subsequently and progressively lost their expression during the first 10 days of pancreatic endocrine differentiation. We also found that miR-200 family miRs, reported as regulators of epithelial-mesenchymal transition, were expressed in native islets, strongly down-regulated during expansion and re-expressed upon pancreatic endocrine differentiation. As for islet-associated miR-375, this was highly expressed in native islets and showed an extreme down-regulation in proliferating hPIDM cells, which progressively re-acquired its expression

during their differentiation into an islet-like phenotype. Of note, during the first 10 days of such differentiation, miR-375 showed an increasing expression parallel to that of insulin gene.

Conclusion: We have identified a specific expression signature of stem- and differentiated islet-associated miRs during expansion and re-differentiation of hPIDM cells, indicating that these miRs may have a role in the regulation of human beta cell mass and islet regeneration phenomena.

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Generation and characterisation of pancreas-specific Tcf7l2 null mice

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Background and aims: Genome wide association studies (GWAS) generate vast quantities of data and identify potential genetic contributions to complex polygenic disorders such as type 2 diabetes (T2D) that would often have been difficult, if not impossible, to predict using a candidate gene approach. However, identifying the causal gene within a particular genetic locus, and understanding the mechanisms and tissues through which the implicated genetic variations act, has been challenging. A single nucleotide polymorphism (SNP) rs7903146 in the TCF7L2 gene was identified in GWA studies as strongly (odds ratio >1.3) associated with the development of T2D. Patients carrying risk alleles of rs7903146 display impaired insulin secretion and decreased sensitivity to the incretin glucagon-like peptide 1 (GLP-1). It has also been reported that islet TCF7L2 message levels are elevated in T2D patients compared to controls whilst TCF7L2 protein content is depressed. These changes were accompanied by down-regulation of GLP-1 and glucose-dependent insulintropic polypeptide (GIP) receptor expression. Our own and others' work has subsequently shown that Tcf7l2 silencing in isolated primary islets and clonal beta cells leads to increased apoptosis and impaired beta-cell function. In the present, study we sought to determine whether deletion of Tcf7l2 selectively in the pancreas in mice may be sufficient to impair glucose homeostasis in vivo.

Materials and methods: Pancreas-specific Tcf7l2 null mice (pTcf7l2 mice) were generated by crossing Tcf7l2-flox^d mice (GenOway) with Cre deleter animals in which Cre recombinase expression is driven by the Pdx1 promoter (a kind gift from Prof. D. Melton, Harvard University). Mice were genotyped by PCR using genomic DNA from ear biopsies, and gene expression in pancreatic islets of Langerhans was assessed by real-time quantitative PCR (qPCR). Glucose tolerance was assessed following intraperitoneal injection or oral administration of glucose (1g/kg). Insulin secretion was assessed in isolated islets during static incubations and assessed by radioimmunoassay.

Results: Tcf7l2 transcript levels were undetectable (Ct > 39) in islets from pTcf7l2 mice vs islets from littermate controls. pTcf7l2 mice displayed normal glucose tolerance and body weight at 8, 12 and 16 weeks, as measured by intraperitoneal glucose tolerance test, but had decreased oral glucose tolerance compared to control littermates (n=7–13 mice from 4 separate litters). Interestingly, Glp1r and insulin2 (Ins2) gene expression were significantly lower in pTcf7l2 islets (42 ± 0.08 % and 15.4 ± 4.6 % decrease, respectively) vs control islets. Islets from pTcf7l2 mice displayed impaired glucose and GLP-1 stimulated insulin secretion (54.6 ± 4.6 % and 44.3 ± 4.9 % lower than from control islets cultured at 16.7 mM glucose and 16.7 mM glucose plus 100 nM GLP-1, respectively), in keeping with published data on human islets, which may be due, at least in part, to the down-regulation of Glp1r expression.

Conclusion: Loss of Tcf7l2 gene expression selectively in the pancreas is sufficient to cause impaired tolerance to oral glucose, whilst responses to intraperitoneal glucose are normal. This likely reflects altered Glp1r expression in beta cells and an impaired response to circulating GLP-1.

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Role of glial cell line-derived neurotrophic factor in endocrine pancreas formation

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Background and aims: Recent studies have suggested that endocrine pancreas formation is regulated by signals from the neural crest cells that migrate and populate the gut with neural and glial cells during embryonic

development. Glial Cell Line-Derived Neurotrophic Factor (GDNF) is a neurotrophic factor that plays a critical role in the development and survival of the enteric nervous system. Both in vitro and in vivo experiments have shown that GDNF overproduction promotes beta cell survival and proliferation. To determine the functional role of GDNF in endocrine pancreas formation in mice we have decided: 1) to analyze the expression pattern of GDNF during pancreas development and 2) to genetically inactivate GDNF in pancreas.

Material and methods: GDNF expression was studied by semiquantitative PCR and by the use of a lacZ-based transgenic mouse throughout embryonic development and adulthood. Cre/lox recombination was used to conditionally eliminate GDNF in mouse pancreatic cells. The pancreata of the mutant mice was analyzed by immunohistochemistry and molecular biology techniques.

Results: GDNF expression was detected at embryonic day 10.5 (E10.5), soon after the pancreas evaginates for the foregut, and E12.5 along with progenitor markers such as Pdx-1 but not in differentiated glucagon producing cells. At E14.5, after the secondary transition, GDNF expression persisted in Pdx-1 expressing progenitor cells in the ducts. From E15.5 on, GDNF was expressed only in differentiating ducts albeit at much lower levels, and after birth became almost undetectable. GDNF was detected exclusively within pancreatic epithelial cells, with no expression in mesenchyme throughout the embryonic development. This is in sharp contrast to other regions of the gut where GDNF is expressed only in mesenchyme. GDNF was specifically inactivated in developing pancreatic epithelium using the Pdx-1-Cre transgenic mouse line. No differences in islet architecture and mass were observed between GDNF knockout mice and control mice, demonstrating the GDNF function is dispensable for islet formation. However, a profound loss of neural cells was found in the pancreata, including islets, of GDNF knockout mice, as determined by staining for the neural marker HuC/D. Our analysis of the embryonic pancreas indicates that migration of neural progenitor cells is compromised in pancreas-specific GDNF mutant mice.

Conclusion: GDNF is expressed in the pancreatic progenitor compartment during early embryonic development becoming restricted to ducts at later stages. The colocalization of GDNF and progenitor markers during embryonic development suggests a functional role for GDNF in embryonic pancreas development. However, our results show that GDNF is dispensable for pancreas formation. Our results indicate that the production of GDNF in embryonic pancreatic epithelium is necessary for the neural colonization of pancreas. We believe the pancreas-specific GDNF mutant mice might be a useful to analyse neural-islet interactions in endocrine pancreas.

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Self-replication is not the only mechanism of maintenance of pancreatic beta cell mass after birth

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Background and aims: Pancreatic beta cell mass is maintained throughout lifetime to control blood glucose levels. Although it has been shown that the major mechanism of the maintenance of beta cell mass after birth is self-replication of pre-existing beta cells, pancreatic beta-cells might also be generated from non-beta-cells. We address this issue by using the inducible Cre/loxP system to trace beta cells.

Materials and methods: We generated Ins2-CreERT2 knock-in mice, in which a fusion protein containing Cre-recombinase (Cre) and modified estrogen receptor is replaced with mouse insulin 2 gene. Unlike conventional transgenic mice expressing Cre driven by insulin promoter, expression of Cre in Ins2-CreERT2 knock-in mice reflects insulin expression with no position effect. We crossbred Ins2-CreERT2 knock-in mice with R26R-YFP mice to generate Ins2-CreERT2/R26R-YFP double knock-in mice, in which pancreatic beta cells can be labeled specifically and permanently upon injection of tamoxifen, and traced the beta-cells by pulse and chase experiment in several different conditions. For tracing beta-cells in adult pancreas, mice of 6 weeks of age were injected with tamoxifen to label the beta cells. For tracing in neonates, pregnant mothers of the mice were injected with a single dose of 4-hydroxytamoxifen on the day before they gave birth. For tracing beta cells after injury, the mice were injected with streptozotocin after tamoxifen treatment. In the chase periods, pancreata removed from the mice were processed for immunostaining. The frequency of labeling of the pancreatic beta cells

was calculated by dividing the number of YFP-positive cells by the number of insulin-positive cells.

Results: When beta cells were labeled in adults under physiological and untreated conditions, $25.8 \pm 3.75\%$ of insulin-positive cells were labeled with YFP and the frequency of the labeling (labeling index) was not altered throughout the 12-month experimental period. In addition, the labeling index was not changed after ablation of beta cells by streptozotocin treatment. In contrast, when tamoxifen was injected to pregnant mothers just before they gave birth, the labeling index in the neonates was decreased significantly around weaning, suggesting that beta cells might be generated from non-beta cells. In addition, small insulin-positive cell clusters, an indication of neogenic islets, containing no YFP-positive cells were detected in this period.

Conclusion: These results indicate that various mechanisms are involved in the maintenance of beta cells after birth and that the present system using knock-in mice is useful for investigation of beta cell fate.

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OP 09 Prediction of complications in type 2 diabetes

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Change in HDL cholesterol and risk of hospitalised coronary artery disease or stroke among patients with type 2 diabetes

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Background and aims: The burden of cardiovascular disease (CVD) is especially high among patients with diabetes. Low levels of high density lipoprotein cholesterol (HDL-C) are associated with CVD, but it remains unclear whether raising HDL-C reduces CVD risk. The aim of this study was to assess whether changes in HDL-C were associated with risk of hospitalization for coronary artery disease or stroke among a population-based sample of patients with type 2 diabetes.

Materials and methods: We conducted an observational cohort study of 30,067 members of Kaiser Permanente who had type 2 diabetes and two HDL-C measurements 6–24 months apart from 2001–2006. After calculating change in HDL-C, we followed patients for up to 7.5 years (through 2009) to determine whether change in HDL-C was associated with CVD hospitalizations. We examined HDL-C change continuously, and also stratified patients into categories of change: increased HDL-C by 6.5mg/dl, decreased HDL-C by 6.5mg/dl, or remained within ± 6.4 mg/dl. Cox regression models were used to assess the association of baseline HDL-C and change in HDL-C with CVD hospitalizations, adjusted for demographic and clinical risk factors as well as comorbidities and pharmaceutical use.

Results: Of the 30,067 study subjects, HDL-C levels remained within ± 6.4 mg/dl for 61.4% (n=18,449), increased by at least 6.5mg/dl for 21.6% (n=6,488), and decreased by at least 6.5mg/dl for 17.0% (n=5,130). Over a mean follow-up of 55.8 ± 23.8 months, 3,717 (12.4%) patients experienced a CVD hospitalization. After multivariable adjustment, each 5mg/dl increase in baseline HDL-C was significantly associated with 7% lower CVD hospitalization risk (HR 0.93, 95% CI 0.92–0.95, $p < 0.0001$) and each 5mg/dl increase in HDL-C change was associated with a 5% CVD risk reduction (HR 0.95, 95% CI 0.93–0.97, $p < 0.0001$). In categorical analysis, a 6.5mg/dl HDL-C or greater decline was associated with 15% increased CVD risk (HR 1.15, 95% CI 1.05–1.27, $p = 0.003$) while a 6.5mg/dl or greater increase was associated with a 10% CVD risk reduction (HR 0.90, 95% CI 0.83–0.98, $p = 0.016$) relative to individuals with stable HDL-C.

Conclusions: In our large, diverse, population-based sample of patients with diabetes, we found that declining HDL-C was positively and significantly associated with risk of CVD hospitalization while increasing HDL-C was significantly and inversely associated with risk of CVD hospitalization. Our study adds to the growing body of evidence suggesting that raising HDL-C levels may be an important strategy for CVD risk reduction and suggests that prevention of HDL-C declines may be equally important.

Table: Fully adjusted hazard ratios of hospitalized CVD associated with baseline HDL-C and change in HDL-C

	Hazard Ratio (95% Confidence Interval)	P value
Continuous Model		
Baseline HDL per 5mg/dl	0.93 (0.92 - 0.95)	<0.0001
HDL-C Change per 5mg/dl	0.95 (0.93 - 0.97)	<0.0001
Categorical Change Model		
HDL-C Remained ± 6.4 mg/dl	1.00	--
HDL-C Decreased < -6.5 mg/dl	1.15 (1.05 - 1.27)	0.003
HDL-C Increased ≥ 6.5 mg/dl	0.90 (0.83 - 0.98)	0.016

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Glycaemic control and heart failure in 83,021 patients with type 2 diabetes

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Background and aims: Intensive glycaemic control has shown no preventive effect in heart failure (HF) in patients with type 2 diabetes. Findings from observational studies are not consistent with respect to the relationship between glycaemic control and HF. The aim was therefore to study the association between glycaemic control and HF in a large study with long follow-up of patients with type 2 diabetes.

Materials and methods: We linked data from patients with type 2 diabetes from the Swedish National Diabetes Register, with the national hospital discharge and mortality registries. The diagnosis of HF is validated as correct in 90–95% of cases. Unadjusted and adjusted incidence rates were estimated by Poisson regression and relative risk by Cox regression.

Results: A total of 83,021 patients with type 2 diabetes, registered between 1998 and 2003 (mean age 65.8 years (SD 11.7) years), and initially free from HF were followed through December, 2009. During a median follow-up of 7.2 years, 10,969 patients (13.2 %) were hospitalized with a primary or secondary diagnosis of HF. The unadjusted incidence rates of HF per 1,000 person-years ranged from 13.8% (95% CI 12.9,14.8) for patients with HbA1c <6.0% (42.0 mmol/mol) to 25.8 % (23.5,28.4) for patients with HbA1c ≥10.0% (85.8 mmol/mol). Cox regression was performed adjusting for age, sex, diabetes duration, smoking, BMI, systolic and diastolic blood pressure, prior or intervening myocardial infarction, atrial fibrillation, heart valve surgery, ischaemic heart disease, use of beta blockers, angiotensin converting enzyme-inhibitors and angiotensin receptor blockers. The adjusted hazard ratio was 2.01 (95% CI 1.79–2.27) for patients with updated mean HbA1c>10.0% (85.8 mmol/mol), as compared with patients with HbA1c <6.0% (42.0 mmol/mol) (reference). The hazard ratio for each percentage unit (10.9 mmol/mol) increase in HbA1c was 1.16 (95% CI 1.14–1.18).

Conclusion: The incidence of HF in type 2 diabetes was high, with 13.2% hospitalized for HF during a mean follow-up period of 7.1 years in a population with a mean age of 65.8 years. Impaired glycaemic control is strongly and independently associated with hospitalization for HF.

Clinical Trial Registration Number: N7A

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Trends in hospital admissions for major cardiovascular events and procedures among people with and without diabetes between 2004 and 2009 in England: a nationwide study

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Background and aims: Despite population reductions in cardiovascular disease (CVD) in recent decades, it is unclear whether this decline is present in both people with and without diabetes. We aimed to compare recent trends in hospital admission rates for angina, acute myocardial infarction (AMI), stroke, percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG) among people with and without diabetes in England.

Materials and methods: We identified all patients aged >16 years with cardiovascular events in England between 2004 and 2009 using national hospital-activity data. Diabetes and non-diabetes specific rates were calculated for each year. To test for time trend, we fitted Poisson regression models.

Results: Over the study period, diabetes-related admission rates for angina, AMI and CABG decreased significantly in England by 5% (rate ratios 0.95 (95% CI 0.94–0.96)), 5% (0.95 (95% CI 0.93–0.97)) and 3% (0.97 (95% CI 0.95–0.98), $P<0.001$ for all) per year. The incidence of stroke did not significantly change in people with and without diabetes (rate ratio 0.99 (95% CI 0.99 (0.98–1.00) and 0.99 (95% CI 0.97–1.01) per year, respectively), while admission rates for PCI increased in both groups (1.02 (95% CI 1.01–1.03, $P<0.01$) and 1.03 (95% CI 1.01–1.05, $P<0.001$) per year, respectively). People with diabetes experienced similar proportional changes for all CVD outcomes as those without diabetes. The median length of hospital stay signifi-

cantly decreased for all admissions in both groups over the study period. Diabetes was associated with an approximate 3.5 to five-fold risk of CVD events. **Conclusion:** This is the first study we are aware of to identify recent diabetes-related admission rates for major CVD events using a national sample that covers the entire population of England. These national data show a considerable decline in hospital admission rates for angina, acute myocardial infarction and CABG, unchanged rates for stroke and an increase in PCI procedure rate among both people with and without diabetes in England. These findings emphasize the ongoing need for aggressive risk reduction and primary prevention of CVD.

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Predicting the mortality of people with type 2 diabetes mellitus following a major complication: a study using Swedish National Diabetes Register data

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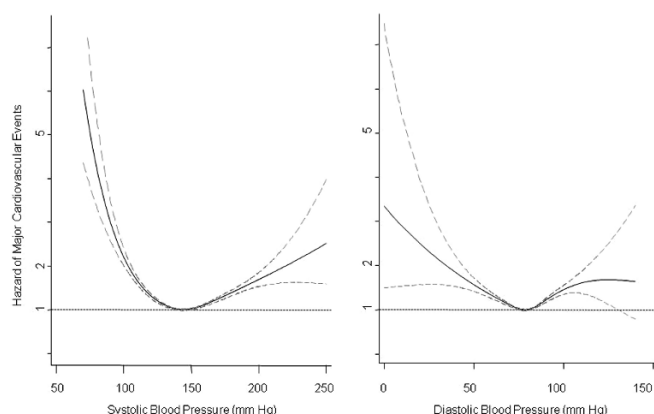
Background and aims: The aim of this observational study was to predict the risk of mortality and life expectancy for diabetic patients following diabetes-related complications.

Materials and methods: This study comprised of people in Sweden with type 2 diabetes alive on or after 1 January 2001. Data were collected from three separate sources: 1) risk factor data from the Swedish National Diabetes Registry (NDR); 2) hospital records of inpatient episodes; and 3) Swedish death records. Data were confidentially linked at the individual patient level by the NDR. The cohort comprised of 22,751 patients who had one of the following complications of diabetes between January 2001 and December 2007: myocardial infarction, stroke, heart failure, amputation or renal failure. Logistic regression was used to estimate mortality within the first month post-complication and a Gompertz proportional hazards model was used to model mortality after the first month post-complication. Follow-up was ended at 31 December 2007. For both models, the following risk factors were examined: type of complication, sex, age at time of complication, duration of diabetes, smoking status, HbA1c, systolic blood pressure, ratio of total cholesterol to HDL, microalbuminuria, proteinuria and Chronic Kidney Disease (CKD) using eGFR.

Results: Besides age at the time of complication and the type of complication, factors increasing the immediate risk of mortality following a complication included: male (OR = 1.10; 95% CI: 1.01, 1.20); duration of diabetes per decade (OR = 1.15; 95% CI: 1.10, 1.21); HbA1c > 8% (OR = 1.27; 95% CI: 1.12, 1.45); smoking (OR = 1.66; 95% CI: 1.48, 2.05); body mass index <20 kg/m² (OR = 1.64; 95% CI: 1.27, 2.11); systolic blood pressure <110 mmHg (OR = 1.55; 95% CI: 1.14, 2.09); proteinuria (OR = 1.14; 95% CI: 1.01, 1.29). These same factors also increased the risk of mortality after the first month: male (HR = 1.14; 95% CI: 1.07, 1.21); duration of diabetes per decade (HR = 1.05; 95% CI: 1.02, 1.09); HbA1c > 8% (HR = 1.13; 95% CI: 1.03, 1.25); smoking (HR = 1.56; 95% CI: 1.41, 1.72); body mass index <20 kg/m² (HR = 1.58; 95% CI: 1.33, 1.89); systolic blood pressure <110 mmHg (HR = 1.55; 95% CI: 1.30, 1.84); proteinuria (HR = 1.33; 95% CI: 1.22, 1.45). CKD was also associated with increased risk of mortality after the first month: moderate decrease in GFR (HR = 1.19; 95% CI: 1.11, 1.28), severe decrease in GFR (HR = 1.41; 95% CI: 1.23, 1.63), and end-stage kidney failure (HR = 1.90; 95% CI: 1.49, 2.43).

Conclusion: In this large, nationwide, population-based study that included a range of risk factors, we have derived a two-part model that can be used to predict survival following five different types of diabetes-related complications. Several risk factors including smoking, BMI, and kidney function were shown to significantly impact on mortality following a major complication. This information is useful in understanding the prognosis of patients following major complications and are useful in modeling the potential benefits of altering risk factors such as smoking status.

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Systolic and diastolic blood pressure levels associated with lowest risk in type 2 diabetes: a cohort study of 37,798 primary care patientsJ. Sundström^{1,2}, C.J. Östgren³, B. Svennblad², R. Sheikhi¹, J. Bodegård⁴, P.M. Nilsson⁵, G. Johansson⁶;¹Department of Medical Sciences, Uppsala University, ²Uppsala Clinical Research Center, Uppsala University, ³Department of Medical and Health Sciences, Linköping University, ⁴AstraZeneca Nordic, Södertälje, ⁵Department of Clinical Sciences, Lund University, Lund, ⁶Department of Public Health & Caring Sciences, Uppsala University, Sweden.**Background and aims:** Aggressive blood pressure treatment targets in type 2 diabetes guidelines have recently been questioned. We therefore aimed to investigate the systolic and diastolic blood pressure levels associated with the lowest risk of cardiovascular events in a large primary care-based sample of patients with diabetes.**Materials and methods:** We identified a cohort of 37,798 patients with type 2 diabetes aged 35 years or older (mean age 65 years) by in 2010 extracting data from electronic patient records for all patients who had a diagnosis of type 2 diabetes or had glucose lowering agents prescribed between 1999 and 2008 in 76 Swedish primary care centers. We followed the cohort for subsequent incidence of a combined endpoint of major cardiovascular events (myocardial infarction, stroke, heart failure, or cardiovascular death) by linking the patients to the Swedish National Cause of Death and Hospital Discharge registries. We investigated restricted cubic spline functions for annually updated systolic and diastolic blood pressures (in separate models) in Cox proportional hazards models, adjusting for age and sex.**Results:** During a median of 3.8 (range 0–10) years of follow-up, 8,994 patients (24%) had a major cardiovascular event (incidence rate 55/1000 person-years at risk). The associations of systolic and diastolic blood pressures with risk of major cardiovascular events were U-shaped (figure). The lowest risk was observed at a systolic blood pressure of 146 mm Hg and a diastolic blood pressure of 79 mm Hg.**Conclusion:** In a large primary care-based sample of patients with type 2 diabetes, associations of systolic and diastolic blood pressures with risk of major cardiovascular events were U-shaped. The blood pressure levels associated with the lowest risk were not consistently in agreement with treatment target levels recommended in current guidelines.

Clinical Trial Registration Number: NCT01121315

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HbA_{1c} as predictor of morbidity and all-cause mortality in people with type 2 diabetes: a Danish population-based observational studyM.V. Skriver¹, H. Støvring², J.K. Kristensen¹, M. Charles¹, A. Sandbæk¹;¹Dept. of General Practice, ²Dept. of Biostatistics, Aarhus University, Denmark.**Background and aims:** The evidence for recommending individuals with type 2 diabetes to strive toward a HbA_{1c} level below a certain value is growing, but there is however sparse evidence from population-based follow-up studies. Since 2003 the Danish Department of Health have recommended offering intensive antidiabetic treatment aimed at reducing HbA_{1c} to less than 7% to all individuals with type 2 diabetes. Thus, the aim of this study is to investigate, in a population-based setting, whether individuals with type 2 diabetes and a HbA_{1c} level below 7% have a lower diabetes related morbidityand/or all-cause mortality than individuals with type 2 diabetes and a HbA_{1c} level above 7%.**Materials and methods:** Individuals with type 2 diabetes were identified with a dedicated validated algorithm, from public data files in Aarhus county, Denmark. The algorithm has a sensitivity of 96% and a positive predictive value of 89%. Individuals were identified in the years 2000 to 2006 and were required to have at least one HbA_{1c} measurement. In total 22,238 people were identified. HbA_{1c} level was defined as the average for each individual of all HbA_{1c} measurements in the year of the first registered HbA_{1c} measurement. Information of morbidity was obtained from the Danish Hospital Discharge Registry (which covers all hospitalisations in Denmark) and information of death and emigration was obtained from the Danish Civil Registration System (which comprises all Danes). Survival was estimated by the Kaplan-Meier method. Possible excess mortality and/or morbidity for individuals with HbA_{1c} above 7% compared with individuals with HbA_{1c} below 7% were estimated with 95% CIs using Cox proportional hazard models. Analysis was stratified on whether information of diabetes duration was observed or not. Adjustment was made for age, sex, prior cardiovascular disease and number of prior admissions of non-cardiovascular diseases. Further adjustment was made for diabetes duration when analysing the subgroup where this information was available. The proportionality assumption was tested using Schoenfeld residuals. Time since 1st of January the year after the first registered HbA_{1c} measurement was used as time scale. Each person was followed until death or morbidity outcome, emigration (censoring) or two years after inclusion, whichever came first. Analyses were performed using Stata version 10.1.**Results:** Please see the table for the specific hazard ratios. In general, a HbA_{1c} level above 7% increased mortality and diabetes related morbidity. Inclusion of diabetes duration slightly lowered the excess risk.**Conclusion:** In this population based study individuals with type 2 diabetes and a HbA_{1c} level below 7% had lower mortality and lower diabetes related morbidity than those with a level above 7%. This supports the recommended treatment target of a HbA_{1c} level below 7%Hazard ratios for individuals with HbA_{1c} ≥ 7%. Individuals with HbA_{1c} < 7% as reference group

		All individuals (N=22,238)		Individuals with information of duration (N=11,701)	
		n	Adjusted HR (95% CI)	n	Adjusted HR (95% CI)
Mortality	All-cause mortality	2286	1.28 (1.18–1.40)	619	1.13 (0.95–1.33)
Morbidity	Arteriosclerosis	170	1.78 (1.28–2.46)	90	1.69 (1.04–2.75)
	Acute compl. to diabetes	208	2.34 (1.70–3.23)	158	1.80 (1.24–2.63)
	Retinopathy	491	2.13 (1.74–2.62)	382	1.68 (1.33–2.12)
	Nephropathy	147	2.96 (1.95–4.50)	122	2.32 (1.45–3.71)
	AMI	542	1.13 (0.94–1.35)	255	1.24 (0.95–1.61)
	Stroke	651	1.05 (0.89–1.23)	287	1.03 (0.81–1.32)
	Neuropathy	147	2.03 (1.40–2.93)	106	1.83 (1.16–2.92)
	At least one of the above	2151	1.46 (1.33–1.60)	1254	1.47 (1.30–1.67)

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OP 10 Exploring mechanisms of insulin resistance

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Pioglitazone improves insulin sensitivity by modulating novel biomarkers: results from the ACTNOW study

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Background: ACT NOW is a randomized double-blind, placebo-controlled study to examine whether pioglitazone (PIO) can prevent/delay development of type 2 diabetes mellitus (T2DM). The aim of the study was to examine the effect of pioglitazone on novel biomarkers that could contribute to the metabolic effects of pioglitazone.

Methods: 602 IGT subjects (FPG =105, 2-h PG [OGTT]=168 mg%) were randomized to PIO (45 mg/day) or placebo (PLAC) and followed for 2.4 years. Indices of insulin secretion and insulin sensitivity were derived from the plasma glucose, insulin, and C peptide concentrations during the OGTT and in a subset from IVGTT before and at study end. Using mass spectrometry, targeted analysis was conducted in 431 individuals with IGT who completed the ACT NOW study. Selected from our previous euglycemic clamp metabolomics studies, we measured top-ranking insulin sensitivity metabolites, including plasma alpha-hydroxybutyrate (alpha-HB), linoleoyl-GPC, oleoyl-GPC, glycine, serine, betaine, decanoylcarnitine, and oleic acid, before and after treatment with pioglitazone/placebo.

Results: 50 PLAC-treated subjects developed diabetes versus 15 PIO-treated subjects (hazard ratio=0.28; 95% CI= 0.16, 0.49; p<0.0001). The improvement in Matsuda insulin sensitivity index in PIO-treated (3.9 ± 0.2 to 7.6 ± 0.3 mcg/ml; p<0.0001) subjects was ~2-fold greater than in PLAC-treated (3.9 ± 0.2 to 5.2 ± 0.3 mcg/ml, p<0.0001 PIO vs PLAC). There was no significant difference between groups in plasma metabolite concentrations at baseline. At study end pioglitazone-treated individuals had significantly lower alpha-HB (3.85 ± 0.1 vs 4.33 ± 0.1 mcg/ml, p=0.002), oleic acid (73 ± 1 vs 82 ± 2 mcg/ml, p=0.001) and higher glycine (15.9 ± 0.3 vs 13.7 ± 0.3 mcg/ml, p<0.005), serine (9.1 ± 0.4 vs 8.2 ± 0.1 mcg/ml, p<0.005), linoleoyl-GPC (13.0 ± 0.3 vs 11.2 ± 0.2 mcg/ml, p<0.005). At baseline matsuda index of insulin sensitivity correlated with alpha-HB (r=0.178, p<0.005), glycine (r=0.296, p<0.005), serine (r=0.123, p<0.005), oleoyl-GPC (r=0.267, p<0.005) and betaine (r=0.157, p=0.001). The improvement in Matsuda index of insulin sensitivity correlated with change in alpha-HB (r=0.112, p=0.02), glycine (r=0.275, p<0.005), serine (r=0.272, p<0.005) and oleoyl-GPC (r=0.144, p=0.04).

Conclusion: Pioglitazone modulates novel metabolites related to lipid metabolism and oxidative stress which may in part explain the beneficial effects of pioglitazone on insulin sensitivity.

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Hyperglycaemia enhances the expression of the diabetogene ped/pea-15 through chromatin remodelling

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Background and aims: Both genes and environment contribute to the development of type 2 diabetes (T2D) and the molecular mechanisms of gene-environment interplay in T2D onset have recently received increased attention. Indeed, emerging evidence indicates that epigenetics plays an important role in the regulation of gene expression and environment may affect chromatin

structure, increasing thus the risk of diabetes in exposed individuals. PED/PEA-15 is a gene commonly over-expressed in T2D subjects and in their offsprings, healthy individuals with a high risk of developing diabetes. In addition, the over-expression of ped/pea-15 gene in mice impairs glucose tolerance and leads to diabetes when they are fed a high-fat diet. The aim of this study was to evaluate whether hyperglycaemia contributes to changes in ped/pea-15 expression and to investigate changes in chromatin structure due to hyperglycaemia and associated with ped/pea-15 expression.

Materials and methods: The rat skeletal muscle cell line L6 was cultured in DMEM supplemented with either 5.5 mM D-glucose (normal glucose, NG) or in 25 mM D-glucose (high glucose, HG). Real Time-Polymerase Chain Reaction (RT-PCR) and Chromatin Immunoprecipitation (ChIP) assays have been performed to evaluate ped/pea-15 expression and changes in chromatin structure at the ped/pea-15 promoter, respectively.

Results: L6 cells cultured in HG showed an increase of the ped/pea-15 mRNA levels up to twofold compared to NG cultured cells. We could exclude the osmotic stress as a cause of this result, since culturing L6 cells under osmotic stress conditions, such as in 20 mM mannitol for 2 weeks, had no effect on ped/pea-15 expression. Time-course experiments revealed an increase of ped/pea-15 mRNA levels already after a 4 hour exposure to HG. We used ChIP experiments to show that HG increased the binding of nuclear factor κB (NF-κB) p65 subunit and the activator protein 1 (AP-1) c-jun subunit to the ped/pea-15 promoter in L6 cells. Furthermore, the HG-induced recruitment of chromatin remodelling factors to the ped/pea-15 promoter was assayed with an antibody specific for the acetyl-histone H3 at lysine 9 and lysine 14, and other epigenetic marks are now under investigation. In addition, in our laboratory new treatments are going on to confirm the importance of NF-κB and AP-1 transcription factors in ped/pea-15 expression, such as treatment with the tumor necrosis factor alpha (TNF-alpha), which is known as a potent inflammatory mediator.

Conclusion: These results provide novel evidence that a close relationship exists between ped/pea-15 gene expression and acetylated histone at the ped/pea-15 promoter under hyperglycaemia conditions. In more detail, our results suggest that hyperglycaemia increases the activation of AP-1 and NF-κB and regulates chromatin remodelling, leading to an increase in ped/pea-15 expression. This may have a role in explaining how hyperglycaemia in turn exacerbate insulin resistance, contributing significantly to the pathogenesis of the disease.

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The role of diacylglycerol concentrations in the development of lipid-mediated insulin resistance in human skeletal muscle

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Background and aims: Skeletal muscle is mainly determines insulin resistance in humans with or at risk of type 2 diabetes, but the underlying cellular mechanisms causing are still unclear. Possible mechanisms are alterations in adipocytokines linked to inflammation, mitochondrial function and intramuscular lipid metabolites [ceramides, diacylglycerols (DAGs)] and have been tested in animal studies, but not yet proven in humans.

Materials and methods: We examined 16 healthy humans (n=16, 30±5 years, BMI: 24±2 kg/m²) and measured circulating cytokines (n=6: TNFα, IL-6, sI-CAM, adiponectin, RBP-4, FGF-21), intramuscular DAG and ceramide contents, protein kinase C isoforms (PKC β, Δ, θ) expression and activities as well as mitochondrial function before and after a 4h infusion of lipid (Intralipid 20%) or glycerol infusion. Detailed mitochondrial function, including ADP-coupled glutamate and succinate oxidation (state 3), FCCP-induced maximal oxidative capacity (state u) as well as kinetics of NADH and FADH₂ dehydrogenases were examined in permeabilized muscle fibers with high-resolution respirometry. Thereafter, a hyperinsulinemic [40 mU/(m².min)]-euglycemic clamp was performed combined with [6,6-2H₂]glucose to assess muscle insulin sensitivity.

Results: Whole-body glucose disposal was ~66% lower during lipid infusion than during glycerol infusion (P<0.000001). Associated with lipid-induced insulin resistance, muscular DAG, but not ceramide, content increased ~2fold (P<0.005) at 2.5h and remained elevated (~1.4fold, P<0.05) at 4 h of lipid infusion. At 4 h, only the activity of PKCθ, but not other isoforms, was raised by 64% (P<0.005). On the other hand, plasma concentrations of all measured cytokines but FGF-21 were not affected despite the marked insulin resistance.

FGF-21 was ~3.5 fold higher at 4 h lipid as compared to glycerol experiments (863 ± 151 vs 248 ± 68 pg/ml, $p < 0.009$). Mitochondrial oxidative capacity and kinetics of respiratory chain complexes I/II also remained unchanged during the lipid infusion.

Conclusion: These results support the concept that DAG-induced activation of PKC θ plays a key role in causing lipid-induced insulin resistance in human skeletal muscle. On the other hand, neither circulating adipocytokines nor muscular ceramides contribute to acute lipid-induced muscle insulin resistance in humans.

Clinical Trial Registration Number: NCT01229059

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Obesity is associated with elevated Hsp60 plasma levels that might contribute to the development of insulin resistance in insulin sensitive cell types

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Background and aims: Obesity is a strong risk factor for the development of insulin resistance and associated with increased circulating pro-inflammatory adipokines. It has been suggested that adipokines are important paracrine mediators of adipose tissue inflammation and endocrine mediators of the crosstalk between adipose tissue and other tissues such as skeletal muscle, contributing to insulin resistance. The signals initiating these processes are not well understood to date, but recent studies characterized stress protein heat shock protein 60 (Hsp60) as a potent inducer of inflammation processes in murine adipocytes. Thus, this study aimed at analysing the inflammatory and metabolic effects of Hsp60 on human adipocytes and skeletal muscle cells (SkMC). Moreover, human adipocytes were investigated as a putative source of circulating Hsp60 and Hsp60 plasma levels from lean and obese individuals were determined.

Materials and methods: Preadipocytes from human subcutaneous adipose tissue and myoblasts were differentiated in vitro. The release of pro-inflammatory mediators after Hsp60 treatment was measured by multiplexbeads assays. Insulin signaling, pro-inflammatory and cellular stress pathways were analyzed by Western blots. The uptake of 2-deoxy-glucose in SkMC was measured for 2 h after insulin (10^{-7} mol/l) administration for 30 min. Hsp60 release was determined by ELISA from adipocytes without and with stimulation by a mixture of cytokines (TNF α , IL-1 β , IFN γ , 1000 U/ml each). Hsp60 plasma levels were determined by ELISA in 18 lean (BMI: 23.4 ± 5.6 kg/m²) and 23 obese (BMI: 44.5 ± 5.6 kg/m²) men.

Results: Hsp60 release from human adipocytes was 2-fold increased after cytokine treatment (0.9 ± 0.3 ng/ml; $p < 0.05$) compared to control (0.4 ± 0.3 ng/ml). In human adipocytes and SkMC, Hsp60 inhibited insulin-stimulated phosphorylation of Akt. Furthermore, Hsp60 exposure decreased glucose uptake in SkMC (1.7 ± 0.1 -fold over basal; $p < 0.01$) compared to control (2.1 ± 0.1 -fold over basal). To elucidate the underlying mechanisms, stress signaling was investigated in both cell types. Hsp60 causes a 2- to 3-fold increase of phosphorylation of ERK1/2, JNK and NF κ B. Moreover, Hsp60 exposure led to increased secretion of TNF α (63.9 ± 49.2 pg/ml; $p < 0.001$), IL-8 (1.2 ± 0.9 ng/ml; $p < 0.01$) and RANTES (970.5 ± 351.0 pg/ml; $p < 0.01$) from human adipocytes, compared to medium control. Furthermore, Hsp60 induced a significant release of MCP-1 (1.2 ± 0.6 ng/ml; $p < 0.001$), IL-8 (1.7 ± 0.9 ng/ml; $p < 0.001$) and IL-6 (0.4 ± 0.3 ng/ml; $p < 0.001$) from SkMC after 24h. Hsp60 plasma levels were 1.6-fold higher in obese than in lean males ($p < 0.05$) and positively correlated with body mass index ($p = 0.04$; $r = 0.34$), plasma leptin ($p = 0.04$; $r = 0.34$) and QUICKI ($p = 0.04$; $r = 0.38$), indices of insulin resistance.

Conclusion: Human adipocytes release Hsp60, which in turn induces secretion of various pro-inflammatory adipocytokines. Hsp60 induces insulin resistance via inflammatory pathways in human adipocytes and SkMC. As circulating Hsp60 levels are elevated in obese humans, Hsp60 likely contributes to obesity-associated inflammation and insulin resistance.

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Reduced syntaxin-5 in skeletal muscle of patients with type 2 diabetes is linked to increased diacylglycerol, activation of PKC θ and impaired insulin signalling

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Chronic mTOR inhibition induces muscle insulin resistance despite weight loss in rats

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Background and aims: mTOR inhibitors are currently used as immunosuppressive therapies in transplanted patients and as promising anti-cancer agents. However, new-onset diabetes is a frequent complication occurring in patients treated with mTOR inhibitors such as rapamycin (Sirolimus).

Materials and methods: We investigated the mechanisms associated with the diabetogenic effects of chronic Sirolimus administration in rats fed either a standard or a high-fat diet. Rapamycin effects on glucose metabolism and insulin signaling were further evaluated in cultured myotubes.

Results: Sirolimus treatment (SIR) for 21 days promoted a decrease in cumulative food intake (-21.1 ± 7.5 %, $p < 0.001$) and concomitant weight loss (delta BW gain: $+35.5 \pm 3.8$ g for CTL vs -7.1 ± 2.1 g for SIR, $p < 0.001$). Compared to pair-fed rats, SIR also induced a specific fat mass loss measured by EchoMRI (-62.9 ± 12.4 %, $p < 0.01$) that was independent from changes in food intake. Despite these beneficial effects, Sirolimus-treated rats were glucose intolerant (AUC of GTT: 878.2 ± 24 for CTL vs 1179.3 ± 74 for SIR, $p < 0.01$), hyperinsulinemic (fasting insulinemia: 1.4 ± 0.2 ng/ml for CTL vs 3.5 ± 0.6 ng/ml for SIR, $p < 0.05$) and hyperglycemic (fasting glycemia: 4.6 ± 0.1 mM for CTL vs 6.4 ± 0.5 mM for SIR, $p < 0.05$), but not hyperlipidemic. Skeletal muscles

represented the major site of Sirolimus-induced insulin resistance as assessed by euglycemic-hyperinsulinemic clamps (GIR in mg/kg/min: 21.6 ± 1.1 for CTL vs 9.3 ± 0.7 for SIR, $p < 0.001$). In vitro, long-term rapamycin treatment of L6 myotubes inhibited insulin-induced glucose uptake (by $62.9 \pm 11.2\%$, $p < 0.001$) and glycogen synthesis (by $96.2 \pm 6.4\%$, $p < 0.001$). At the molecular level, both in vitro and in vivo analyses showed that rapamycin (Sirolimus) impairs muscle glucose metabolism by preventing full insulin-induced Akt activation and by altering the expression of glucose transporters. Finally, rats fed a high-fat (HF) diet and treated with Sirolimus displayed an exacerbate glucose intolerance (AUC of GTT: 1223.4 ± 89 for HF fed rats and 1789.0 ± 141 for HF fed rats + SIR, $p < 0.01$) although they were protected from diet-induced obesity.

Conclusion: Taken together, our data demonstrate that the diabetogenic effect of chronic rapamycin administration is due to an impaired insulin action on glucose metabolism in skeletal muscles.

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OP 11 Education

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Individualised diabetic education can contribute to decrease the incidence of diabetic foot and avoid amputation: results of a 9-year prospective study

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Background and aims: The annual morbidity of diabetic foot ulcers (DFU) is between 1.2% and 3.1% and 49.5% and 27.4% separately in elder or pre-elder patients. 33% of patients with DFU needed to be amputated in China. There are more of the changeable factors including patient education, optimizing glycaemic control, smoking cessation, and diligent foot care etc. There is some dispute about significance of diabetic education on prevention and prognosis of diabetic foot ulcers.

Materials and methods: 229 patients with high-risk diabetic foot (with diabetic neuropathy or Lower extremity vascular disease or with both) were randomly divided into individual education group ($n=125$)(IDE) and conventional educational group ($n=104$)(CDE). They were separately given the individual diabetic education and conventional diabetic education on the basis of the original therapeutic schedule. The difference of cumulative incidence, amputation rate, mortality, ABI and vibration threshold value were observed every year from January 1999 to December 2009.

Results: 1. The annual incidence of diabetic foot ulcer IDE group was lower than that in CDE group from the third year ($P < 0.05$). The cumulative incidence of diabetic foot ulcers in IDE Group at 9 years was 19.2%, RDE group was 39.4%. The relative risk decreased 51.3%. 2. By the end of ninth year, ABI were separately 0.75 ± 0.09 (IDE Group) 0.51 ± 0.10 (CDE group), (IDE vs CDE, $P < 0.001$). 3. The vibration threshold value was separately 16.9 ± 1.7 volts (IDE), 29.5 ± 1.4 volts (CDE) (IDE vs CDE, $P < 0.05$). 4. At the sixth year end, there was significant difference between the two groups whether or amputation rate of mortality ($P < 0.05$). At the end of ninth year, the relative risk of amputation in IDE group decreased 39.95%, $P < 0.01$, the relative risk of death decreased 40.52%, $P < 0.05$.

Conclusion: Individualized diabetic education may not only decrease the incidence of diabetic foot ulcer, but also retard the progress of diabetic foot and reduce amputation rate and mortality.

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The effect of self-monitoring of blood glucose on metabolic control in type 2 diabetes patients

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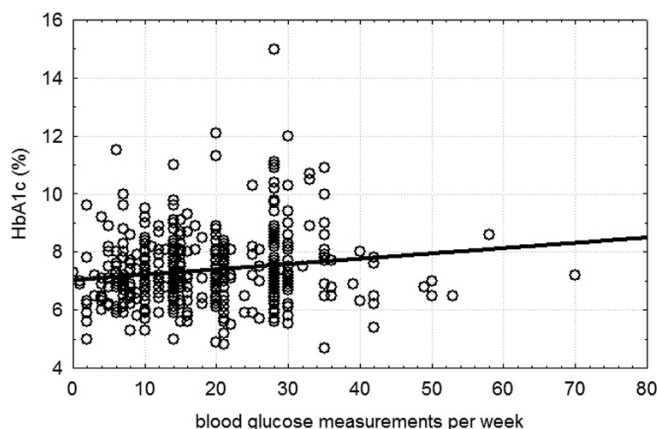
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Background and aims: The role of self-monitoring of blood glucose (SMBG) in type 2 diabetes treatment remains controversial, and the effect of SMBG on the medication dosage adjustment in type 2 diabetes is unclear. We conducted a multi-centre cross-sectional study assessing the relationship between application of SMBG and the glycemic control.

Materials and methods: The study group comprised 564 patients with type 2 diabetes (mean age [\pm SD] 64.3 ± 9.9 years, diabetes duration 10.4 ± 8.4 years, BMI 31.3 ± 5.5 kg/m², 59.7% treated with insulin, HbA_{1c} $7.37 \pm 1.26\%$, fasting and postprandial blood glucose 128 ± 27 and 150 ± 34 mg/dl, respectively). The patients were asked to fill in a standardized questionnaire consisting of closed questions regarding their use of SMBG.

Results: Mean frequency of SMBG was 2.9 ± 1.3 /day and 18.1 ± 10.2 /week. The patients who performed SMBG 3 or more times/day as compared to SMBG < 3 times/day had longer duration of diabetes (11.5 ± 9.0 vs 9.2 ± 7.5 years, $p < 0.01$), higher HbA_{1c} (7.63 ± 1.33 vs $7.28 \pm 1.10\%$, $p < 0.01$), were taking insulin more times daily (3.1 ± 1.2 vs 2.4 ± 1.1 injections/day, $p < 0.001$) and were treated with higher daily insulin dose (56 ± 31 vs 44 ± 26 IU, $p < 0.001$). More frequent SMBG showed slight but significant positive correlation with HbA_{1c} ($r=0.144$, $p < 0.05$; see figure) and insulin daily dos-

age ($r=0.229$, $p<0.05$). However, multiple regression analysis showed that only BMI and not frequency of SMBG was an independent determinant of HbA_{1c} . Patients performed SMBG “to know their blood glucose” (76%), “per doctor’s order” (67%), and “to know what to eat” (53%). Antidiabetic medication dosing was modified according to SMBG results by 46% of subjects. Pre-meal elevated blood glucose resulted in food intake decrease in 51%, and increase in physical exercise in 55% of the studied subjects. **Conclusion:** SMBG does not seem to be associated with improved metabolic control in type 2 diabetes - probably the opposite is true i.e. patients with worse glucose control tend to perform SMBG more often. As only half of subjects change their behavior due to abnormally high results of SMBG, therefore patients with type 2 diabetes should be educated more effectively to utilize SMBG to modify their lifestyle.



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Group follow-up compared to individual clinic follow-up after structured education for type 1 diabetes: the Irish DAFNE Study

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Background and aims: To compare the effectiveness of an education-based strategy of group follow-up with a traditional individual clinic visit strategy after participation in the Dose Adjustment for Normal Eating (DAFNE) structured education programme for patients with type 1 diabetes.

Materials and methods: We cluster randomised 6 hospital diabetes clinics across Ireland. Participant eligibility included type 1 diabetes for more than 1 year and a HbA_{1c} below 13 percent. Group follow-up was delivered at 6 and 12 months post-DAFNE using a semi-structured curriculum with an emphasis on meeting the perceived needs of the group and goal setting. Control arm patients received individual clinic visits from a doctor, nurse and/or dietitian. Change in HbA_{1c} and rates of severe hypoglycaemia were assessed at 6, 12 and 18 months.

Results: Mean duration of diabetes among participants ($n = 437$) was 16 years; mean HbA_{1c} was 8.3 percent with over 29 percent having a HbA_{1c} below 7.5 percent and 24 percent reporting one or more episodes of severe hypoglycaemia in the previous year. At 18 months there was no clinically or statistically significant change in mean HbA_{1c} in either group and no between-group difference in mean HbA_{1c} over time (0.11%, 95% CI (-0.22 to 0.44), $p=0.41$). Among patients with baseline HbA_{1c} above 7.5 percent we found no significant difference between groups (0.1%, 95% CI (-0.31 to 0.51), $p=0.53$) but mean HbA_{1c} did improve over time (-0.16%, 95% CI (-0.27 to 0.06), $p<0.001$). Rates of severe hypoglycaemia decreased significantly over time (0.27 episodes per patient per year, $p<0.001$) but there was no difference between groups. Episodes of severe hypoglycaemia per patient were categorised as ‘increased’, ‘no change’ or ‘decreased’ and an analysis based on proportions was undertaken. This provided convincing evidence that, regardless of treat-

ment ($p=0.89$), those with no hypoglycaemic episodes at baseline tended not to worsen over the period of the study as 90% remained hypoglycaemia free (95% CI (84%, 94%)) while 10% (95% CI (6%, 16%)) reported an increase. Of those that had 1 or more hypoglycaemic episodes at baseline 90% reported a decrease (95% CI (78%, 97%)), 4% reported an increase (95% CI (0.005%, 13%)) while 6% reported no change (95% CI (1%, 17%)) with no evidence of a significant treatment effect ($p=0.28$).

Conclusion: In a relatively well controlled cohort of patients with type 1 diabetes DAFNE training was associated with no change in mean HbA_{1c} over 18 months but with significant improvement in rates of severe hypoglycaemia. The addition of group follow-up for 12 months following DAFNE training was not associated with improvement over and above that seen with one-to-one clinic visits. Our data do not support implementation of group-based care for individuals with type 1 diabetes.

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Effectiveness of therapeutic-educational re-training in patients affected by type 1 diabetes treated with CSII (continuous subcutaneous insulin infusion)

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Background and aims: Treatment of type 1 diabetes (T1DM) using insulin pumps has certainly made a significant improvement in metabolic control and patients quality of life. However, it is not rare to see a gradual stabilization of the metabolic advantage gained at the beginning of therapy and the progressive deterioration of the glycometabolic situation in some cases. Considering that the role of medical nutrition therapy (MNT) and education of advanced Continuous Subcutaneous Insulin Infusion (CSII) function is not standardized after the initial phase of CSII installation, we performed an educational re-training to patient with long standing insulin pump to test its effectiveness in the metabolic control.

Materials and methods: We enrolled 23 T1DM patients treated with CSII at least for 12 months. The subjects were randomized into two homogeneous groups: a control group $n=11$ (mean age 34 ± 15) and treatment group $n=12$ (mean age of 39 ± 15) and they were followed for six months. Either groups were subjected to quarterly diabetes visits with blood tests and continuous glucose monitoring for 3 days. The treatment group, received in addition six educational weekly group meetings regarding the technical re-training on advanced features of the insulin pump, a healthy diet, the insulin/carbohydrate ratio, carbohydrate counting, insulin sensitivity, index and glycemic load.

Results: Baseline HbA_{1c} levels were similar in treatment group and control group (8.1% vs 8.3% $p=ns$), at sixth month HbA_{1c} levels in treatment group showed a significant reduction compared to control group (7.1% vs 8.3%, $p=0.03$ and $p=0.04$ vs baseline). Furthermore, patients in treatment group showed a significant lower glucose variability at sixth month measured by continuous glucose monitoring compared to control group ($p=0.004$). No significant differences between the two groups in terms of hypoglycemia and weight gain were observed.

Conclusion: Educational training made patients more aware of their disease and more autonomous in the management of everyday problems. Cycles of educational group meetings can be considered an effective therapeutic tool in the maintenance or improvement of metabolic control in patients on insulin pump therapy.

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Effect of diabetes self-management education combined with self-monitoring of quantitative urine glucose on glycaemic control in non-insulin-treated type 2 diabetes

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Background and aims: To assess whether diabetes self-management education (DSME) combine with self monitoring of urine glucose (SMUG) or self monitoring of blood glucose (SMBG) is effective, convenient and safe for glycaemic control in non-insulin treated type 2 diabetes.

Materials and methods: This is a prospective, randomized, controlled pilot study. Adults with non-insulin treated type 2 diabetes were recruited from two clinical centers and randomized into three groups: SMBG by using a blood glucose meter (LifeScan OneTouch[®] Ultra Easy[™], n=38), SMUG by using a quantitative urine glucose meter (TANITA[®] UG - 201, n=35), and the control group without self-monitoring (n=35). All patients accepted 5 days standard diabetes education for diabetes knowledge and self-management skills, and were followed up for six months at four weekly intervals, during which identical diabetes care and laboratory measurements were provided.

Results: There was a significant decrease of glycated haemoglobin (HbA_{1c}) within each group (p<0.05). At end-point, mean changes (95% confidence interval) in HbA_{1c} from baseline were -1.9% (-2.5, -1.3) for SMUG group, -1.5% (-1.9, -1.1) for SMBG group and -1.0% (-1.9, -0.2) for the control group, and proportions of patients achieving HbA_{1c} ≤6.5% were 38.9%, 35.3% and 20.0% respectively. However, no significant differences between groups were found. The average monitoring frequency was significantly higher in SMUG group than in SMBG group (74.5±11.6 vs. 58.6±14.6 times per month, p<0.05). Overall hypoglycaemia incidences and scores of diabetes quality of life were similar between groups, although DQOL scores increased significantly only in SMUG and SMBG groups (p<0.05).

Conclusion: This pilot study suggests that DSME combined with SMUG has comparable efficacy with SMBG on glycaemic control while incorporated into diabetes education, life style interventions and pharmacological treatment. Furthermore, SMUG with a quantitative urine meter owes better compliance than SMBG, without influencing the quality of life or risk of hypoglycaemia. Supported by: Key Program of Jiangsu Natural Science Foundation (BK2010087)

were significantly decreased compared to professional group after providing education. The study subjects of professional group had significantly better DBP (mmHg, 81.8±13.1 vs 87.2±8.2, p<0.006) compared to peer leader group. No significant differences were found in SBP (mmHg, 132.6±11.7 vs 129.1±12.6, p=0.5) and BMI (kg/m², 25.0±2.8 vs 22.8±9.3, p=0.3) of the study subjects between peer leader and professional group.

Conclusion: These findings suggest that education with peer support may have improved desired glycemic achievement on diabetes management among type 2 diabetic persons.

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Effectiveness of diabetes education on improving glycaemic achievement by professionals versus peers

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Background and aims: Diabetes is a complex disorder that requires a life-long process of self-care, demanding life-long treatment and lifestyle changes. Diabetic patients should be made aware of the sign and symptoms, consequences of the disease. Studies related to education with peer support for improving glycaemic status among diabetes subjects are relatively rare in developing countries like Bangladesh. The aim of the study was to compare the effectiveness of diabetes education with professionals vs peer leaders for achieving target glycaemic status in type 2 diabetic subjects.

Materials and methods: A total of 133 type 2 diabetic subjects (HbA_{1c} >7%) were screened purposively from the OPD of BIRDEM (the tertiary hospital of Diabetic Association of Bangladesh) under a follow-up study. Sixty seven participants led by 4 professionals (diabetes educator) versus 66 participants led by 4 peer leaders. For both groups (4 professionals + 4 peer leaders) total 3 education classes, 4 hours/per class was conducted with a predesigned curriculum. Changes over after 12 weeks educational intervention as well as psychological support and related variables (FBG, HbA_{1c}, weight, SBP and DBP) were compared on 124 (n=59 for professional vs n=65 for peer leader) type 2 diabetic subjects (mean ±SD, age 53.4±10.4 yrs; M 64%, F 36%). Two women and two men were selected as peer leaders who had type 2 diabetes (within last 5 years), education level up to graduation, HbA_{1c} <7%, having in-depth knowledge of disease and good interaction capacity with others. Differences between baseline and follow-up data were calculated using paired t-test and unpaired t-test were used for group differences.

Results: Fasting blood glucose (mmol/L, 7.07±1.9 vs 5.3±2.1, p<0.001) and HbA_{1c} (%), 10.6±1.2 vs 8.04±1.1, p<0.04) level of the study subjects were significantly reduced among peer leader group after getting education intervention. SBP was also significantly reduced (mmHg, 140.7±17.5 vs 132.6±11.1, p<0.002) though no significant difference was found before and after value of DBP (mmHg, 85.1±9.2 vs 88.0±7.8), weight (kg, 68.7±11.1 vs 68.10±10.4) and BMI (kg/m², 25.2±3.05 vs 25.09±2.8) in this group. The level of HbA_{1c} (%), 9.51±1.7 vs 9.05±1.5, p<0.001) of the study subjects were significantly reduced after getting education intervention by professionals. However, after receiving education no significant difference was found in SBP (mmHg, 134.6±16.7 vs 129.4±13.7), DBP (mmHg, 82.6±10.6 vs 83.4±7.9), FBG (mmol/L, 7.9±3.2 vs 6.7±0.8) and BMI (kg/m², 24.7±5.9 vs 23.8±8.04) values of this group. Fasting blood glucose (mmol/L, 3.9±3.1 vs 6.8±1.7, p<0.0001) and HbA_{1c} (%), 8.04±1.1 vs 9.1±1.5, p<0.0001) level of the study subjects in peer leader group

OP 12 Vascular actions, new and established therapies

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Glucagon-like peptide-1 receptor activation increases myocardial blood flow but does not alter myocardial glucose uptake or metabolism in patients with type 2 diabetes

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Background and aims: Cardiovascular effects of glucose-lowering agents are of increasing interest. The incretin hormone glucagon-like peptide-1 (GLP-1) and GLP-1 receptor (GLP-1r) agonists currently emerging as antidiabetic medications have shown promising cardiovascular effects and are speculated to have cardioprotective potentials. Patients with type 2 diabetes mellitus (T2DM) have altered metabolism in the heart with reduced glycolysis and increased beta oxidation of free fatty acids. Thus, a mechanism of enhancing myocardial energetic efficiency by increasing glucose availability and utilization and thereby reducing beta oxidation and increasing cardioprotection may exist. Of interest, GLP-1 seems to increase myocardial glucose uptake (MGU) in dogs. Thus, our aim was to assess the effects of GLP-1r activation on glucose metabolism and blood flow in hearts of subjects with T2DM.

Materials and methods: We conducted a randomized, double-blinded, placebo-controlled cross-over study including eight type 2 diabetes patients, all males, insulin naive, and without coronary artery disease. Positron emission tomography was used to determine the effect of GLP-1 on myocardial blood flow (MBF) and myocardial glucose uptake (MGU) during a pituitary-pancreatic hyperglycemic clamp (plasma glucose 9.0 mmol/l) with ¹³N-ammonia and ¹⁸fluoro-deoxy-glucose (FDG) as tracers. We used a 3-compartment model to describe MGU and MBF. Since exenatide does not seem to have any acute hemodynamic effect on heart rate or blood pressure in humans (1; 2), we used this GLP-1r agonist.

Results: While GLP-1r activation did not affect MGU (0.13 ± 0.07 and 0.15 ± 0.05 micromol/g/min, P=0.66), GLP-1r activation increased MBF from 0.73 ± 0.094 to 0.85 ± 0.091 ml/g/min, p=0.0056. The efflux rate of FDG from cardiomyocytes to blood was 0.88 ± 0.3 min⁻¹ and 1.15 ± 0.2 min⁻¹ (P=0.53) and the amount of FDG metabolised in cardiac mitochondria was 0.04 ± 0.02 min⁻¹ and 0.04 ± 0.02 min⁻¹ (P=0.93), indicating that GLP-1r activation did not alter glucose uptake nor change the intracellular glucose phosphorylation rate. Except from changes in C-peptide levels, no differences in circulating hormones and metabolites (catecholamines, free fatty acids, insulin, growth hormone, cortisol and P-glucose) were found.

Conclusion: The phosphorylation rate of glucose in myocardial cells seems to be extremely low in patients with T2DM and is not altered by exenatide. While GLP-1r activation does not seem to enhance MGU, the myocardial muscle blood flow however increases acutely after infusion of exenatide. Surprisingly, this is not induced by increases in catecholamines, thus we speculate a direct action through the GLP-1r. In spite of increase in blood flow the MGU did not change pointing to a decrease in glucose extraction rate.

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Exendin-4 directly improves endothelial dysfunction in obese rats through cAMP/AMPK-eNOS pathways

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Background and aims: There is growing evidence to suggest that Glucagon-like peptide-1 (GLP-1) may play an important role in the cardiovascular system. Some human and animal studies indicated that infusion of GLP-1 has beneficial effects on endothelial function. The aim of this study was to determine whether GLP-1 per se, independent of hormonal changes, might improve endothelial dysfunction in aorta isolated from high-fat diet induced obese rats.

Materials and methods: Male wistar rats 5 weeks of age were randomized to receive a regular chow (Control, n=5) or a high-fat supplemented chow

(OB, n=10). The rats were sacrificed after 10 weeks feeding and thoracic aorta was dissected and cut into four rings of 3-mm length. The response to acetylcholine (ACh) and sodium nitroprusside (SNP) were examined in organ bath. In order to study the direct effects of exendin-4 (a GLP-1 agonist) on obese rats vascular function and investigate possible mechanisms of action of exendin-4, the aortic rings obtained from obese rats were sub-divided into four groups and incubated in organ bath with exendin-4 (2.5nM) in the presence or absence of the following specific inhibitors of candidate pathways: (1) 20μM AMPK inhibitor compound C (Ex-4+CC, n=10), (2) 200mM adenylate cyclase inhibitor Dideoxyadenosine (Ex-4+D, n=7), (3) 1mM NO synthase inhibitor L-NAME (Ex-4+L-NAME, n=10), and (4) vehicle (Ex-4, n=10). After 1 hour of incubation, the aortic rings were precontracted with norepinephrine (0.1μmol/L), then the rings were exposed to cumulative concentration of Acetylcholine (ACh, 10⁻⁹-10⁻⁵M) or sodium nitroprusside (SNP, 10⁻¹⁰-10⁻⁶M) to test the endothelial dependent (EDV) and independent vasodilation (EIV).

Results: Acetylcholine caused a concentration dependent vascular relaxation in all pre-constricted aortic rings. The maximum EDV value was (94.6±1.9)% in the control group and (53.4±5.8)% in the OB group (p < 0.05). The EIV values were comparable between two groups. Pre-incubation of obese rats vessels with exendin-4 significantly increased cumulative relaxation to ACh, the maximum EDV value increased from (53.4±5.8)% to (76.3±5.5)% (p < 0.05). The beneficial effect of exendin-4 on obese rats EDV was partly attenuated in the presence of AMPK inhibitor (Ex-4+CC: 52.1±3.6%, p < 0.05), adenylate cyclase inhibitor (Ex-4+D: 58.8±5.3%, p < 0.05), and NO synthase inhibitor (Ex-4+L-NAME: 58.1±6.6%, p < 0.05). SNP induced vessels relaxation had no statistical significance among four groups.

Conclusion: Endothelial function was impaired in obese rats. The current study demonstrated that exendin-4 directly mitigate impaired endothelial-dependent vasorelaxation of isolated obese rats aortas. The beneficial effect of exendin-4 appears to be mediated, at least in part via stimulation of cAMP/AMPK-related signal transduction pathway and enhancement of eNOS activity. The directly endothelial action of exendin-4 indicates potential therapeutic benefit beyond glycaemic control in obese diabetic patients.

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Exendin-4 protects human coronary artery endothelial cells against lipoperoxidation

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Background and aims: Inappropriate apoptosis of endothelial cells is an important mechanism behind endothelial dysfunction, which plays an important role in macrovascular complications in diabetes. We recently showed that the glucagon-like peptide-1 receptor is expressed in human coronary artery endothelial cells (HCAEC) and that GLP-1 improves endothelial dysfunction in type 2 diabetic patients with coronary artery disease. The aim of the present study was to investigate the role of the stable GLP-1 receptor agonist exendin-4, which has been approved for clinical use against type 2 diabetes, on apoptosis of HCAECs under hyperlipidemic conditions *in vitro*.

Materials and methods: HCAEC cells were cultured in the endothelial growth medium in the presence of 5 mM glucose. Phosphorylation and expression of endothelial nitric oxide synthase (eNOS), p-38 Map kinase and JNK were examined by Western blotting using anti-phospho-eNOS, p-38, JNK and anti-eNOS, p-38 and JNK antibodies, respectively. Alpha-tubulin examined in same blots acted as loading control. Caspase-3 activity and DNA fragmentation were evaluated using ELISA kit as a measure of apoptosis after 24 h-incubation with the reagents.

Results: Incubation of the cells with 0.125 mM palmitate provoked apoptosis, and this effect was significantly inhibited by exendin-4 or GLP-1 (7-36). In contrast, palmitate-induced apoptosis was not affected by the GLP-1 metabolite GLP-1(9-36). Incubation of HCAEC cells with palmitate alone for 24 h resulted in an increased eNOS, p-38 MAP kinase and JNK phosphorylation, which were neutralized by exendin-4. The protective effect of exendin-4 on apoptosis was prevented after treatment of the cells with the specific inhibitors for protein kinase A or for eNOS. In addition, the effects of exendin-4 on apoptosis and enzyme activation were completely blocked by the GLP-1 receptor antagonist exendin (9-39).

Conclusion: Our study reveals that exendin-4 and GLP-1(7-36) protect HCAECs from lipoperoxidation, an effect that is mediated through the GLP-1 receptor and involves PKA, eNOS, and p-38 MAP kinase, JNK dependent

pathways. These novel findings add yet other beneficial properties of exen-4, increasing its clinical utility in type 2 diabetic patients in whom endothelial dysfunction is a salient feature that adversely affect their survival. Elucidation of the mechanisms underlying these beneficial vascular effects of exen-4/GLP-1 may be exploited when intervening pharmacologically against vascular dysfunction in diabetes and may form the basis of improved incretin enhancers that hold the potential to prevent vascular lesions in diabetic patients.

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Effects of SAR184841 (30 mg/kg/d) on microvascular reactivity in spontaneously hypertensive rats: comparison with irbesartan and rosiglitazone

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Background and aims: Selective 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1) inhibitors have received a major interest for their potential in treating diabetes and macrovascular complications but nothing has been reported yet about their effects on microvascular complications. The aim of this study was to evaluate the effects of chronic oral administration with SAR184841, a selective rodent and human 11 β HSD1 inhibitor, on microvascular reactivity in spontaneous hypertensive rats (SHR) by measuring post occlusive reactive hyperemia (PORH) using the technique of Laser Doppler flowmetry. The effects of SAR184841 were compared with those of the oral antidiabetic rosiglitazone (ROSI) and the antihypertensive drug irbesartan (IRBE).

Materials and methods: 12-week old male SHR (n=48) and their control Wistar Kyoto (WKY, n=12) were included in the study. SHR were treated orally during 21 days with Vehicle (VHC) (0.6% Methocel/Tween 80 in water (0.5 / 99.5, v/v), SAR184841 (30 mg/kg/day), ROSI (5 mg/kg/day) or IRBE (30 mg/kg/day). WKY were treated with VHC (10ml/kg/day). At the end of the treatment period, PORH was assessed in anaesthetized animals using a hyperaemia index measured following the occlusion of the femoral artery. Blood and organ collection were performed to measure insulinemia, biochemical markers and hepatic triglycerides.

Results: In SHR, the hyperaemia index was significantly lower than in WKY (0.20 ± 0.09 versus 0.77 ± 0.18 respectively, $p=0.006$). ROSI was not able to significantly improve microvascular function whereas IRBE and SAR184841 significantly improved the hyperaemia index compared to SHR VHC (0.78 ± 0.09 , $p=0.005$ and 0.72 ± 0.15 , $p=0.034$ versus 0.20 ± 0.09 respectively). Furthermore, both IRBE and SAR184841 restored microvascular function close to the one observed in WKY (0.77 ± 0.18). At the end of the experiment, non fasting insulin blood levels were not changed either between normotensive animals and SHR or among all SHR groups. Conversely, corticosterone levels were found significantly higher in SHR VHC compared to WKY (271 ± 13 and 209 ± 20 ng/mL respectively, $p=0.014$). None of the treatments studied were able to significantly modify corticosterone blood levels when compared to SHR VHC. Similarly adiponectin was found significantly higher in SHR VHC compared to WKY (63.4 ± 7.4 μ g/mL and 38.1 ± 6.2 μ g/mL respectively, $p=0.0096$) but none of the treatments modified these levels significantly. Hepatic TG levels were significantly increased in SHR VHC compared to WKY (13.1 ± 1.7 mg/g versus 3.5 ± 0.5 mg/g respectively, $p<0.001$) and decreased by ROSI treatment vs SHR VHC ($p=0.006$) whereas IRBE treatment did not modify liver TG levels. A clear tendency to decrease hepatic TG levels in SHR was observed with SAR184841 (7.7 ± 1.2 and 13.1 ± 1.7 mg/g respectively) although this effect was not significant ($p=0.055$).

Conclusion: SAR184841 (30 mg/kg/d) significantly improved microvascular reactivity in SHR. In this model, SAR184841 was shown to be equipotent to IRBE on microvascular function and to ROSI on metabolic parameters. These promising results emphasize the potential of 11 β HSD1 inhibitors for the treatment of diabetic microvascular complications.

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Aspirin resistant subjects present a proinflammatory milieu with an increased oxidative stress: in these conditions, high glucose fails to influence platelet responses to agonists

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Background and aims: We previously demonstrated that: i) exposure of platelets from healthy subjects to "in vitro" high glucose attenuates the anti-aggregating effect exerted by aspirin incubation, owing to oxidative stress-mediated inhibition of the aspirin-induced increase of platelet nitric oxide; ii) exposure to "in vitro" high glucose of platelets from "in vivo" aspirin-treated non diabetic patients enhances platelet responses to agonists, but only in aspirin-sensitive patients. To clarify the biochemical mechanisms of this phenomenon, we aimed to clarify the differences between aspirin-sensitive and aspirin-resistant subjects potentially justifying the lack of high glucose effects in the last ones.

Materials and methods: Non-diabetic subjects (n=59, M/F: 33/26, age: 60.2 ± 1.1 years) taking 100 mg/day of aspirin owing to the presence of cardiovascular risk factors and/or previous cardiovascular events, were evaluated for aspirin sensitivity by Platelet Function Analyzer-100 (PFA-100). In aspirin sensitive and aspirin resistant subjects we evaluated: i) platelet aggregation (expressed as Maximal Aggregation, MA) in platelet-rich plasma (PRP) stimulated by sodium arachidonate (NaA, 1mmol/l), ADP (10 μ mol/l) and collagen (4 mg/l) in the presence or the absence of a 60-min preincubation with 25 mmol/l glucose; ii) serum levels of the stable thromboxane metabolite TXB₂; iii) urinary levels of 11-dehydro-TXB₂; iv) a circulating pattern of inflammatory and anti-inflammatory markers including: PCR, IL-1b, IL-1ra, IL-4, IL-6, IL-10, TNF-alpha; v) *in vivo* markers of oxidative stress: 8-OH-2'-deoxyguanosine (8-OH-dG) levels and superoxide dismutase (SOD) activity.

Results: Aspirin resistance was detected in 14 subjects (24%), who, in comparison to aspirin sensitive subjects, showed increased platelet responses to agonists, being MA (%) in response to NaA 14.8 ± 3.5 vs 7.6 ± 6.0 ($p<0.005$), in response to ADP 83.9 ± 7.8 vs 65.0 ± 4.2 ($p<0.04$) and in response to collagen 48.0 ± 10.1 vs 33.9 ± 3.9 ($p<0.02$). In aspirin-resistant subjects platelet exposure to high glucose did not modify aggregating response to agonists, that was reduced in aspirin-sensitive subjects ($p=0.01$ - 0.05 for the different agonists). Furthermore, aspirin resistant subjects showed: i) increased serum concentrations of TXB₂ (pg/ml): 5920.7 ± 1297.8 vs 450.3 ± 72.9 ($p<0.0001$); ii) increased urinary concentrations of 11-dehydro-TXB₂ (ng/mg creatinine): 5.1 ± 0.5 vs 3.5 ± 0.2 , ($p<0.001$); iii) decreased concentrations of anti-inflammatory cytokines (pg/ml) IL-4 (36.5 ± 5.0 vs 87.0 ± 9.3 , $p<0.004$) and IL-10 (27.2 ± 3.8 vs 55.3 ± 2.6 , $p<0.0001$); iv) increased plasma concentrations of 8-OH-dG (ng/ml) (84.8 ± 8.0 vs 31.3 ± 15.5 , $p<0.01$) and decreased plasma SOD activity (U/ml) (4.8 ± 0.14 vs 6.3 ± 0.16 , $p<0.0001$).

Conclusion: Aspirin resistant subjects present not only an incomplete inhibition of COX-1, but also a proinflammatory milieu and an increased oxidative stress, together with an insensitivity to the pro-aggregating effect of glucose. These results suggest that an elevated oxidative stress masks the glucose effects on platelets, that are mediated by the oxidative stress itself.

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Determinants of the variability in the recovery rate of platelet cyclooxygenase activity during chronic therapy with low-dose aspirin in type 2 diabetes

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Background and aims: Aspirin is currently recommended for cardiovascular prevention in type 2 diabetes (T2DM). However, primary prevention trials failed to substantiate its efficacy in T2DM and incomplete platelet inhibition has been reported. Hyperglycemia has been often hypothesized to contribute to such an incomplete inhibition. We tested whether a faster recovery of platelet COX-1 activity and/or a suboptimal 24-hour glycemic control would account for an incomplete thromboxane (TX) A₂ inhibition by low-dose aspirin during the 24-hour dosing interval.

Materials and methods: One-hundred T2DM patients on chronic low-dose aspirin (100 mg daily) were studied. Serum TXB₂, an index of platelet COX-1 activity, was measured every 3 hours, between 12 and 24 hours after a witnessed aspirin administration, to characterize the kinetics of platelet COX-1 recovery (phase 1); 46 out of 100 patients underwent 24-hour continuous glucose monitoring (CGMS) as well. Thirty-three patients with the steepest COX-1 recovery slopes were subsequently randomized to receive aspirin 100 mg daily, 200 mg daily or 100 mg twice daily for 28 days (phase 2). On day 29, COX-1 recovery was reassessed over the 12 to 24 hour dosing interval.

Results: COX-1 activity displayed linear kinetics with large inter-individual variability in recovery slope. Multiple linear regression showed that mean platelet volume, higher BMI quartiles and age predicted serum TXB₂ recovery slope, independently of other variables. None of the CGMS variability indices, (mean 24h glycemic value, standard deviation, MAGE and CONGA1-5) as well as HbA1c and fasting glucose correlated with serum TXB₂ recovery slopes. A twice-daily aspirin regimen, but not a doubling of the dose, completely corrected the abnormal recovery slope of platelet COX-1 activity.

Conclusion: We conclude that inter-individual variability in the recovery rate of platelet COX-1 activity during the aspirin dosing interval most likely reflects abnormal megakaryopoiesis associated with T2DM while is not influenced by 24-hr glucose control or other glycemic indices; inadequate TX inhibition can be overcome by a twice daily regimen.

OP 13 GLP-1 receptor agonists

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Adding insulin detemir (IDet) to liraglutide and metformin improves glycaemic control with sustained weight reduction and low hypoglycaemia rate: 52 week results

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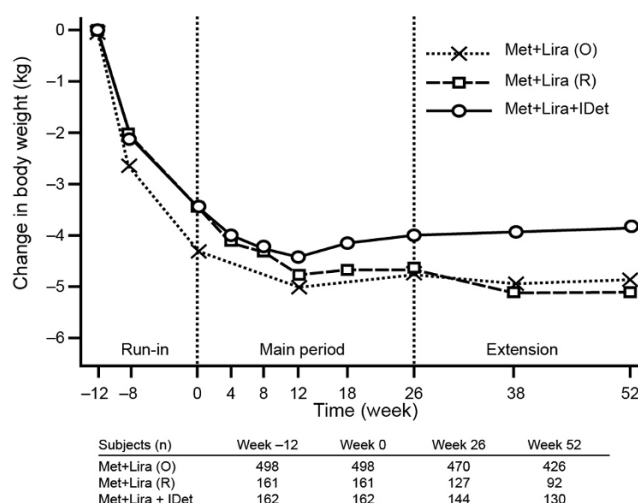
Background and aims: To evaluate and compare efficacy and safety of metformin + liraglutide 1.8 mg + insulin detemir (Met+Lira+IDet) versus metformin + liraglutide 1.8 mg (Met+Lira) in a 26-week extension to the previous 12 week run-in followed by 26-week randomised main trial, 64 weeks in total.

Materials and methods: After the 12-week Met+Lira run-in, subjects with HbA_{1c} <7% entered an observational (O) arm and continued on Met+Lira for 52 weeks. Subjects with HbA_{1c} ≥7% were randomised 1:1 to Met+Lira (R) or Met+Lira+IDet for 52 weeks. IDet was titrated from an initial dose of 10U/day. At weeks 26 and 38 post-randomisation, Met+Lira (O or R) subjects with HbA_{1c} ≥8% could intensify therapy with IDet. Twenty-four Met+Lira subjects (7 of 498 observational and 17 of 161 randomised subjects who entered the main period) intensified with IDet during the extension; their last observation carried forward (LOCF) values before intensification were included in the analyses.

Results: 821 of 988 subjects completed the run-in; of these, 498 (61%) reached HbA_{1c} <7% and proceeded to the Met+Lira (O) arm. The remaining 323 (39%) subjects were randomised. Randomised subjects had a mean screening HbA_{1c} of 8.3%, which decreased to 7.6% following run-in. HbA_{1c} decreased a further -0.50% with Met+Lira+IDet vs. +0.01% with Met+Lira (R) by week 52 post-randomisation; estimated treatment difference (ETD) [95% CI]: -0.51% [-0.70; -0.31]; p<0.0001. By week 52, 52% of Met+Lira+IDet subjects reached HbA_{1c} <7% vs. 22% of Met+Lira (R) subjects; odds ratio [95% CI]: 3.94 [2.37; 6.55]; p<0.0001. All groups lost weight during run-in with a mean of -3.5 to -4.3 kg (Fig). From randomisation to week 52, weight did not increase (-0.05 kg) with Met+Lira+IDet and further decreased (-1.02 kg) with Met+Lira (R); ETD [95% CI]: 0.97 [0.04; 1.91]; p=0.0416. Rates of minor hypoglycaemia were low at 0.12, 0.03, and 0.23 events/subject-year for Met+Lira (O), Met+Lira (R), and Met+Lira+IDet, respectively. No major hypoglycaemic episode occurred in any group from randomisation to week 52.

Conclusion: Intensification of Met+Lira with IDet provided improved glycaemic control with low risk of hypoglycaemia and sustained weight loss for up to 52 weeks.

Mean change in body weight over time, no imputation.



Values after intensification are not included in this figure for the 24 Met+Lira subjects who intensified with IDet during the extension.

Clinical Trial Registration Number: NCT00856986

Supported by: Novo Nordisk

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Four weeks treatment with liraglutide reduces insulin dose without loss of glycaemic control in type 1 diabetic patients with and without residual beta cell function

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Background and aims: To investigate the effect of 4 weeks treatment with liraglutide on insulin dose and glycaemic control in type 1 diabetic patients with and without residual beta-cell function.

Materials and methods: Ten type 1 diabetic patients with residual beta-cell function (C-peptide positive) and nineteen without (C-peptide negative) were studied. All C-peptide positive patients were treated with liraglutide plus insulin, whereas C-peptide negative patients were randomised to liraglutide plus insulin or insulin monotherapy. Continuous glucose monitoring with identical food intake and physical activity was performed before (week 0) and during treatment (week 4). Differences in insulin dose, HbA_{1c}, time spent with blood glucose (BG) <3.9 mM, >10 mM and 3.9–9.9 mM and body weight were evaluated.

Results: Insulin dose decreased from 0.50±0.06 to 0.31±0.08 U/kg/day, p<0.001 in C-peptide positive patients and from 0.72±0.08 to 0.59±0.06 U/kg/day, p<0.01 in C-peptide negative patients treated with liraglutide, but did not change with insulin monotherapy. HbA_{1c} decreased in both liraglutide treated groups. The % reduction in daily insulin dose was positively correlated with beta-cell function at baseline and two patients discontinued insulin treatment. In C-peptide positive patients, time spent with BG <3.9 decreased from 3.0 to 1.0 hour, p=0.03. 18/19 patients treated with liraglutide lost weight during treatment (mean -2.3±0.3 kg, range: -0.5 to -5.1 kg, p<0.001). Transient gastrointestinal side-effects occurred in almost all patients treated with liraglutide.

Conclusion: Treatment with liraglutide in type 1 diabetic patients reduces insulin dose with improved or unaltered glycaemic control.

Clinical Trial Registration Number: NCT00993720

Supported by: Poul and Erna Sehested Hansen Foundation

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Efficacy and safety of exenatide once weekly versus liraglutide in subjects with type 2 diabetes (DURATION-6): a randomised, open-label study

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Background and aims: To compare exenatide once weekly (EQW) versus liraglutide once daily (Lira) on glycemic control, body weight and safety in type 2 diabetes patients treated with life style modification and oral antihyperglycemic medications (OAMs).

Materials and methods: 26-week, multicenter, open-label, randomized (stratified by sulfonylurea use, baseline HbA_{1c} (<9.0%; ≥9.0%) and country), 2-arm parallel study in 19 countries, comparing EQW (2 mg, once-weekly injection) versus Lira (1.8 mg, once-daily injection) in addition to patients' OAMs (metformin, sulfonylurea and/or pioglitazone). Non-inferiority was concluded if the upper limit of the confidence interval for the treatment difference of change from baseline HbA_{1c} (EQW - Lira) was <0.25%.

Results: Of the 912 randomized subjects, 911 subjects were included in the intent-to-treat analysis (450 Lira, 461 EQW). Baseline characteristics (mean [SD]): Age 57 (10) y; BMI 32.3 (5.5) kg/m²; HbA_{1c} 8.5 (1.0) %. Change in HbA_{1c} at endpoint was greater in subjects taking Lira (-1.48%, SE 0.05) than in those taking EQW (-1.28%, 0.05; treatment difference 0.21%, 95% CI (0.08, 0.34) using mixed model repeated measures analysis and the difference did not meet the non-inferiority criteria. More subjects taking Lira achieved HbA_{1c} <7% (n=271, 60.2%) than those taking EQW (n=241, 52.3%) p=0.008. Subjects taking Lira lost more weight (-3.58 kg, SE 0.18) than those taking EQW (-2.68 kg, SE 0.18; treatment difference 0.90 kg, 95% CI [0.40, 1.41]). There was no major hypoglycemia during the study. Minor hypoglycemia was experienced by 50 (10.8%) EQW-treated subjects and 40 (8.9%) Lira-treated subjects (p = 0.374 for treatment difference). Subjects taking Lira and EQW

had similar decreases in systolic and diastolic blood pressure (SBP; -3.5 and -2.5; DBP; -0.5 and -0.5, respectively). Changes in other cardiovascular biomarkers (lipids, high sensitivity C-reactive protein, brain natriuretic peptide) were similar between groups at endpoint. The most common adverse events were gastrointestinal side effects for both Lira-treated subjects (nausea n=92 [20.4%], diarrhea n=59 [13.1%], vomiting n=48 [10.7%]) and EQW-treated subjects (nausea n=43 [9.3%], diarrhea n= 28 [6.1%], vomiting n=17 [3.7%]). Among subjects taking Lira, 24 (5.3%) discontinued due to adverse events (nausea n=8 [1.8%], diarrhea n=3 [0.7%], vomiting n=4 [0.9%]) compared to 12 (2.6%) of subjects taking EQW (nausea n=1 [0.2%], diarrhea n=1 [0.2%], vomiting n=1 [0.2%]).

Conclusion: Both treatment groups demonstrated robust glycemic lowering with associated weight loss. HbA_{1c} lowering and weight loss were greater with daily injections of Lira while gastrointestinal side effects and withdrawals due to adverse events were lower with EQW. Compared to the 5 previous DURATION studies, the efficacy results for EQW in this study were of lower magnitude (range of LS mean HbA_{1c} response -1.5% to -1.9%).

Clinical Trial Registration Number: NCT01029886

Supported by: Eli Lilly and Company and Amylin Pharmaceutical, Inc.

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Safety and efficacy of once monthly exenatide administration over 20 weeks in patients with type 2 diabetes

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Background and aims: Exenatide, a GLP-1 receptor agonist, improves glycaemic control and reduces body weight when administered twice daily or once weekly by subcutaneous (s.c.) injection in patients with type 2 diabetes (T2DM). A new formulation, exenatide suspension, utilizes the extended-release microspheres of exenatide once weekly (ExQW) with a triglyceride-based diluent that enables delivery of higher doses with less frequency. The aim of this trial was to investigate the safety and efficacy of monthly administration of exenatide suspension.

Materials and methods: The safety and efficacy of 3 exenatide once monthly (ExQM) suspension doses (5, 8, or 11 mg s.c.), with ExQW (2 mg s.c.) as a reference arm, were assessed in this randomized, open-label, controlled study with 121 patients (36% female, age 50±10 years, weight 97±19 kg, HbA_{1c} 8.5±1.2%, fasting plasma glucose [FPG] 10.3±2.5 mmol/L, diabetes duration 6±5 years, mean±SD) with T2DM treated with diet/exercise, metformin (MET), pioglitazone (PIO), or MET+PIO. Patients were treated for 20 weeks and received 5 monthly ExQM injections or 20 weekly ExQW injections. Primary analyses were performed at week 20.

Results: Patient retention was high (94%). Sustained plasma exenatide concentrations within the known therapeutic range were achieved with all doses of ExQM. Based on the longer dosing interval, greater peak-to-trough variability was observed with ExQM than ExQW; however, mean trough concentrations of exenatide remained within the therapeutic range across all 3 ExQM doses. The 2 highest ExQM doses achieved exenatide levels similar to ExQW. As seen with ExQW, ExQM concentrations approached non-detectable levels 8 weeks after last injection. HbA_{1c} and FPG were substantially reduced with all doses of ExQM, with improvements comparable to ExQW (Table). Weight loss was observed in all treatment groups. Results were consistent between Evaluable and Intent-to-Treat (ITT) Populations. ExQM was generally well-tolerated with no unique safety findings relative to ExQW. The most frequent adverse events (AE) were headache (17–27%) and nausea (17–23%) for ExQM, and headache (30%) and diarrhea (27%) for ExQW (ITT Population). No major or minor hypoglycaemia was observed. One AE of vomiting with ExQM led to withdrawal. There was no evidence of prolonged AE duration with ExQM or ExQW.

Conclusion: Monthly dosing with exenatide suspension was well-tolerated with robust improvements in glycaemic control in patients with T2DM, supporting further development of the monthly suspension formulation.

Treatment	N	Δ HbA _{1c} (%)	HbA _{1c} <7% at Week 20	Δ FPG (mmol/L)	Δ Weight (kg)
ExQW (2 mg)	29	-1.5 (0.2)	48%	-1.9 (0.5)	-1.4 (0.6)
ExQM (5 mg)	26	-1.3 (0.2)	50%	-1.4 (0.4)	-1.1 (0.8)
ExQM (8 mg)	28	-1.3 (0.3)	57%	-1.7 (0.6)	-0.4 (0.6)
ExQM (11 mg)	27	-1.5 (0.2)	70%	-2.7 (0.5)	-1.1 (0.7)

Changes are expressed as mean (SEM) Δ from baseline to Week 20, Evaluable Population

Clinical Trial Registration Number: NCT01104701

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Long-term, injection-free treatment with ITCA 650, a continuous subcutaneous delivery of exenatide via DUROS[®] device, leads to stable glycaemic and weight control for 48 weeks in metformin-treated type 2 diabetesK. Luskey¹, J. Rosenstock², T. Alessi¹, R.R. Henry³;¹Intarcia Therapeutics, Hayward, ²Dallas Diabetes and Endocrine Center,³University of California at San Diego, La Jolla, USA.

Background and aims: Current therapy with exenatide requires frequent self-injections and is associated with significant nausea, leading to poor therapeutic compliance. An alternative form of exenatide delivery was evaluated in a phase 2 study with ITCA 650, a subcutaneous osmotic delivery system that provides for continuous delivery of exenatide at specified doses for 3 months.

Materials and methods: Subjects (n=155) treated with metformin only and having HbA_{1c} between 7% and 10% were enrolled in a 24-week study to evaluate ITCA 650. Initially subjects were randomized to three groups receiving either ITCA 650 at 20 or 40 mcg/day or exenatide injections at 10 mcg BID. At week 12, subjects were randomized such that subjects treated with ITCA 650 remained on the same dose or were dose escalated to 60 or 80 mcg/day and subjects treated with exenatide injections were treated with ITCA 650 at 40 or 60 mcg/day. At week 24, subjects were offered the option to continue treatment at their current dose of 20, 40, 60 or 80 mcg/day for an additional 24 weeks.

Results: ITCA 650 treatment resulted in significant reductions in HbA_{1c} and weight after 24 weeks. At week 24, 86 subjects (85% of eligible subjects) elected to continue treatment at their current dose. Changes in HbA_{1c} and weight observed over the 48-week treatment, as well as the proportion of subjects with HbA_{1c} <7%, are shown in the table below. The reductions in HbA_{1c} and weight at week 48 were maintained from those initially observed at week 24. Overall, 71% of the subjects had both a reduction in HbA_{1c} and no weight gain. In addition, desirable trends in lipids, blood pressure and heart rate were observed, especially at the higher doses. Changes observed at 60 mcg/day, the chronic dose selected for future Phase 3 studies based on activity and tolerability, were total cholesterol -11.3±31.8 mg/dL, triglycerides -7.4±110.3 mg/dL, LDL-C -10.1±28.3 mg/dL (p<0.05), HDL-C +0.1±7.2 mg/dL, systolic BP -1.7±8.1 mmHg, diastolic BP -7.8±12.5 mmHg and heart rate -1.1±11.4 bpm. Continuing treatment with ITCA 650 was very well tolerated with minimal side effects and a completion rate of 85%. Among all of the subjects, onset of nausea was reported in only one subject and vomiting was reported in another subject throughout the final 24 weeks of treatment. No clinically meaningful change in calcitonin was seen in any subject.

Conclusion: This extension study shows that long-term treatment with ITCA 650 is effective in controlling glycemic parameters and weight and can also lead to potential positive cardiovascular benefits through beneficial changes in lipids and blood pressure. In addition, tolerability is excellent with minimal GI side effects noted with long-term treatment that provides constant, continuous exposure to exenatide. Phase 3 will evaluate devices of 6-12 month duration.

HbA_{1c} and Weight Changes at Week 48

ITCA 650 Dose (mcg/day)	n	Baseline HbA _{1c}	Week 48 HbA _{1c}	Change in HbA _{1c} at Week 48	Subjects with HbA _{1c} ≤7%	Change in Weight (kg)
20	13	7.8±0.8	6.8±0.7	-1.0±0.5*	79%	-2.7±2.3
40	22	7.9±0.8	6.8±0.9	-1.1±1.1*	68%	-4.9±6.6*
60	22	8.1±0.8	6.6±0.7	-1.5±1.0*	78%	-3.5±6.3**
80	13	8.0±1.0	6.6±1.3	-1.4±0.8*	82%	-3.6±6.3***

*p<0.0005 **p<0.01 ***p<0.05

Clinical Trial Registration Number: NCT00943917

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Liraglutide promotes weight maintenance and additional weight loss in obese adults without diabetes after diet-induced weight loss: the SCALETM 56-week randomised studyV.C. Woo¹, P.A. Hollander², K.D. Niswender³, S. Klein⁴, L.J. Aronne⁵, C.B. Jensen⁶, T.D. Le Thi⁶, T. Wadden⁷;¹University of Manitoba, Winnipeg, Canada, ²Baylor University MedicalCenter, Dallas, ³Vanderbilt University School of Medicine, Nashville,⁴Washington University School of Medicine, St. Louis, ⁵Weill-CornellMedical Center, New York, USA, ⁶Novo Nordisk A/S, Søborg, Denmark,⁷Center for Weight and Eating Disorders, University of Pennsylvania School of Medicine, Philadelphia, USA.

Background and aims: Weight loss is difficult to achieve and sustain by lifestyle changes alone. Liraglutide is a once-daily human glucagon-like peptide-1 analogue approved for the treatment of type 2 diabetes mellitus. Here we present data from a randomised, phase 3, placebo-controlled, double-blind trial in obese adults without diabetes that tested the ability of liraglutide (3 mg) to maintain initial diet-induced weight loss and to promote further weight loss.

Materials and methods: Individuals (age ≥18 years, BMI ≥30 or ≥27 kg/m² with comorbidities) who lost ≥5% body weight during a 4-12 week run-in comprising low-calorie diet (1200-1400 kcal/d) and exercise counseling were randomised to receive s.c. liraglutide (N=212) or placebo (N=210) and a 500 kcal/d deficit diet + exercise counseling for 56 weeks. Of 511 individuals entering run-in, 422 (77%) were randomised 1:1 to each arm. Maintenance of run-in weight loss and additional weight loss were the primary endpoints evaluated.

Results: Mean run-in weight loss for all individuals who were randomised was 6.0% (6.3 kg). After 56 weeks of treatment, liraglutide provided statistically significant improvements in all measures of weight loss change from run-in compared to placebo treatment (Table) (p<0.0001 in all analyses). During treatment, net weight changes post-run-in were -6.1% vs 0.1% (-5.7 kg vs +0.2 kg) for liraglutide vs placebo, respectively. Significantly more liraglutide vs placebo recipients maintained run-in weight loss and lost additional run-in weight. More than twice as many participants on liraglutide lost ≥5% additional run-in weight compared to those on placebo. Completion rates, serious AEs and withdrawals due to AEs were similar for each group. More nausea and vomiting were reported during liraglutide vs placebo treatments, occurring mainly during dose-escalation; 64% of liraglutide nausea cases were mild, and most cases declined in frequency by 4-6 weeks. Psychiatric AEs were reported by 11% and 12% of subjects in each arm, respectively.

Conclusion: Liraglutide 3 mg was superior to placebo in maintaining diet-induced weight loss and in achieving additional clinically relevant weight loss in a majority of individuals while demonstrating safety and overall tolerability similar to placebo.

Full analysis set	Liraglutide N=207	Placebo N=206
Post-run-in* weight change, % (kg) ^a	-6.1 ^c (-5.7) ^c	-0.1 (0.2)
Maintained run-in weight loss, % ^b	81 ^c	49
Lost ≥5% body weight, % ^b	51 ^c	22
Safety analysis set	N=212	N=210
Subjects with AEs, n (%)	194 (91.5)	186 (88.6)
Total	9 (4.2)	5 (2.4)
Serious		
Withdrawals, n (%)	53 (25.0)	64 (30.5)
Total	18 (8.5)	18 (8.6)
From AEs		
Nausea, %	47.6	17.1
Vomiting, %	17	2

^aANCOVA, ^blogistic regression, ^cp<0.0001

*Run-in weight loss was 6.0% (6.3 kg)

Clinical Trial Registration Number: NCT00781937

Supported by: Novo Nordisk A/S

OP 14 Diabetes in childhood

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Islet autoantibody testing and prevention of diabetic ketoacidosis

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Background and aims: At diagnosis of type 1 diabetes (T1D), children may suffer potentially fatal ketoacidosis (DKA). The rates in Colorado have recently increased from <30% to >40% despite community education efforts. Screening for pre-diabetes and early treatment could prevent DKA.

Methods: Healthy infants with immediate family history of T1D (n=1,120) or without, but high-risk HLA-DR/DQ genotypes (n=1422), have been followed for up to 16 years. Autoantibodies to insulin, GAD65, IA-2, and ZnT8 have been assessed annually. Persistent autoantibodies have developed in 181 children and T1D in 71. DKA rate at diagnosis was compared in this prospectively followed group (pre-T1D; age 8.4±3.9y; 48%F; 11% minorities) with that in 1101 children diagnosed during the same period in the community (8.3±3.8y; 46%F; 25% minorities).

Results: During the study period, 3 children died at diagnosis of diabetes in the community vs. none in the pre-T1D group. DKA affected 411/1101 (37%) of the community controls and 5/71 (7%) of pre-T1D children (p<0.001). Of the 5 DKA cases in among pre-T1D children, one was 10 mo old and three were not seen by the study for 5–11 years prior to diagnosis. Four additional children were lost to follow-up for 2–11 yrs, yet avoided DKA. Thus DKA developed in 2/64 (3%) of the active study participants and 3/7 (43%) of those who have dropped out. The median HbA_{1c} at diagnosis in these groups was 6.6% (interquartile range 6.2–8.8%) and 12.4% (10.1–12.8%), respectively, compared with 11.2% (9.7–13.4) in the community cases.

Conclusion: Annual screening of high-risk children for islet autoantibodies and counseling of parents of children with pre-T1D can prevent most of the morbidity associated with T1D onset. The benefit is lost with a longer interruption of the follow-up.

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Haemoglobin A_{1c} as an indicator of type 1 diabetes in pediatric and adolescent youth

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Background and aims: HbA_{1c} testing has been recommended by the 2009 International Expert Committee to be used for diagnostic purposes for diabetes. The current recommended thresholds are ≥6.5% for diagnosis of diabetes and ≥5.7% (ADA) for high-risk for progression to diabetes. The aim of this study is to evaluate the sensitivity and specificity of HbA_{1c} as a marker for type 1 diabetes (T1D) and/or impaired glucose tolerance (IGT) in high-risk children and adolescents as compared to the ADA OGTT criteria for diabetes and determine if differences exist by age.

Materials and methods: The study population consisted of youth <21 years of age from the prospective DPT1, TEDDY, TRIGR and TrialNet studies that had an HbA_{1c} within 90 days of a diagnostic 2-hour plasma glucose measure by OGTT to determine sensitivity and specificity. Asymptomatic T1D cases were included in the TEDDY and TRIGR studies since OGTTs were not routinely done. An OGTT ≥200mg/dl or FPG ≥126mg/dl defined diabetes and OGTT 140–199mg/dl defined IGT. ROC analysis was used to assess diagnostic accuracy of the HbA_{1c} test vs. the OGTT (AUC=0.5, poor diagnostic; AUC=1.0, excellent diagnostic).

Results: There were 587 youth from the DPT1, 884 from TrialNet, 102 from TEDDY and 426 from TRIGR included in the analysis. Seventy-five percent of youth were between 5–15 years of age. On applying a threshold of ≥6.5% for T1D diagnosis, the sensitivity was very poor and specificity was excellent (Sensitivity/Specificity: DPT1-24%/98%, TrialNet-28%/99%, TEDDY-34%/98%, TRIGR-39%/100%). ROC analyses suggested that HbA_{1c} was not a good indicator of T1D (0.90 required for diagnostic tests) in the DPT1 (AUC=0.72), TrialNet (0.85), TRIGR (0.60) and TEDDY (0.80) studies. The threshold of ≥5.7% as an indicator of T1D was poor with a higher

sensitivity and lower specificity vs. ≥6.5% threshold (DPT1- 73%/54%, TrialNet- 66%/91%, TEDDY-68%/93%, TRIGR- 46%/89%). HbA_{1c} of 5.7% was also a poor indicator of IGT; sensitivity (8–33%) and specificity were variable (64–95%) across the studies. No difference in HbA_{1c} performance in youth younger vs. older than 10 was noted.

Conclusion: HbA_{1c} ≥6.5% is a specific but not sensitive indicator of T1D in high-risk subjects <21 years of age. Asymptomatic high-risk children with HbA_{1c} ≥6.5% may be diagnosed with T1D without performing an OGTT.

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Urinary C-peptide creatinine ratio is a practical outpatient tool in identifying MODY from type 1 diabetes in children with diabetes

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Background: The young-onset hyperglycaemia seen in patients with maturity onset diabetes of the young (MODY) is frequently misdiagnosed as Type 1 diabetes (T1D), and inappropriately treated with insulin. Measurement of blood C-peptide can aid in the discrimination of diabetes subtypes but has practical limitations. Urinary C-peptide creatinine ratio (UCPCR) is stable at room temperature for 72 hours in boric acid preservative, offering the potential to be a non-invasive alternative to differentiate MODY from T1D in the outpatient setting.

Aims: To assess whether UCPCR can discriminate MODY from Type 1 diabetes in children with diabetes.

Materials and methods: We measured 2 hour postprandial UCPCR in patients <18years with T1D (n=96) and genetically confirmed MODY (n=29; HNF1A/4A n=10, GCK n=19). Samples were collected at home and posted directly to the laboratory for analysis

Results: T1D were similar to MODY patients for age (median(interquartile range) 12.9(10.4–15.4) v 14.6(12.1–16.1)y, p=0.19) and diabetes duration (3.6 (1.2–7.4) v 2.6 (1.5–4.8)y, p=0.28). UCPCR was lower in T1D than MODY: (median (IQR) 0.05(<0.02–0.67) v 3.41 (1.94–4.66)nmol/mmol; p<0.0001). Receiver Operating Characteristic Curves showed excellent discrimination (area under curve 0.95) and identified a cut-off UCPCR ≥1.4nmol/mmol for discriminating MODY from T1D with 100% sensitivity and 85% specificity. 14/95 (15%) T1D patients had UCPCR ≥1.4nmol/mmol and all were diagnosed within 2.5 years of diagnosis.

Conclusion: In children with diabetes, a single post meal UCPCR which may be posted gives excellent discrimination of T1D from MODY and is a useful adjunct to select patients for diagnostic genetic testing.

Supported by: Diabetes UK

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Centre differences in metabolic control in 1133 children with T1DM below 11 years: insulin regimen or centres' recipe?

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Background: The Hvidoere Study Group (HSG) has demonstrated persisting center differences in metabolic control in adolescents in 4 continents. These differences appear to be not attributable to specific insulin regimens but seem to be influenced by centers effectiveness in implementing treatment regimens. To evaluate this in prepubertal children, HSG performed a cross sectional study in children <11 years with T1DM.

Methodology: All children, <11 y with a diabetes duration ≥ 1 y, were invited to participate. CRF's included information on clinical characteristics, treatment, DKA (hospital admission needed), hypoglycaemia (loss of consciousness/seizures), language difficulties and co morbidities. HbA_{1c} was measured centrally by ¹⁸Tosoh liquid chromatography (DCCCT aligned, range 4.4–6.3%).

Results: In total 1133 children from 18 centers participated ([female]: 47.7 %; mean age 8.0 ± 2.1 y; mean diabetes duration 3.8 ± 2.1 y). The grand mean HbA_{1c} was 8.0 ± 1.0 % without significant impact of diabetes duration, age or gender. Language difficulties had an adverse effect ($p = 0.036$) on HbA_{1c}. Significant ($p < .000$) center differences were demonstrated with mean HbA_{1c} varying between 7.3 ± 0.8 and 9.0 ± 1.1 %. Different insulin regimen were used (CSII: 32.8 %, Basal bolus 16.9 %; Conventional : free mix : 36.5 %; premix : 6.3 %; freemix+ (mainly 1 center : extra insulin for snacks/meals), 7.5 %). Significantly lower HbA_{1c} was observed in freemix+ (7.3 ± 0.8 %) and higher in the premix group (8.5 ± 1.7 %). Significant center differences ($p < .000$) in blood glucose measurement (BGM) frequency were reported (2.5 to $8.3 \times$ /day) with a higher frequency in CSII treated and younger children. A significant ($r = -.170$, $p < .000$) inverse correlation was seen with HbA_{1c} and BGM frequency.

Conclusion: In summary, center differences in metabolic outcome are already present in children <11 years, unrelated to diabetes duration, age, or gender, and despite generally lower HbA_{1c} values. BGM frequencies differ between centers and do have a weak effect on the HbA_{1c} level. Although treatment regimen has some effect, the centers effectiveness (recipe) using a specific treatment strategy remains the key factor for the outcome, confirming previous observations.

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Sensor augmented pump therapy from onset of type 1 diabetes: late follow-up results of the Pediatric ONSET Study

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Background and aims: In the European multicentric randomised Pediatric Onset Study evaluating sensor-augmented pump therapy throughout the first year of type 1 diabetes (T1D), we found that children and adolescents with frequent use of sensors had significantly lower HbA_{1c} values at 12 months of treatment than those with less frequent or without sensor use. Aim of the present follow-up study was to evaluate the metabolic control and beta-cell function one year after the end of the intervention.

Materials and methods: 131 of 154 study patients (85.5% follow-up compliance) were re-examined 24 months after T1D onset (49.6% boys, age at onset 8.9 ± 4.3 years). 62 patients had been randomised to the primary group applying a sensor-augmented pump system (Paradigm REAL-Time, Medtronic MiniMed Inc) during the 1st year of T1D, whereas 69 patients belonged to the control group performing conventional insulin pump therapy (CSII) with self-monitoring blood glucose only. Groups were not significantly different concerning age or gender distribution. Additionally to the clinical assessment, HbA_{1c} as well as fasting blood glucose and C-peptide values were centrally measured.

Results: At 24 months, i.e. one year after the end of the interventional study, 50.0% of the patients used the sensor-augmented pump system, 48.3% conventional CSII and 1.7% multiple daily injections. HbA_{1c} was 7.6 ± 1.3 % in the primary group and 7.7 ± 1.2 % in the control group ($p = .493$). Frequent sensor use during the first year of T1D was associated with lower HbA_{1c} values at 24 months as compared with irregular or even no sensor use (7.4 ± 1.0 % vs 7.7 ± 1.3 %), however, this difference did not reach statistical significance ($p = .236$). Although fasting C-peptide was not clearly different between the primary and control group (0.13 ± 0.17 vs 0.09 ± 0.10 nmol/L, $p = .121$), patients with initially frequent sensor use had significantly less C-peptide loss within 24 months (C-peptide reduction 0.02 ± 0.18 vs 0.07 ± 0.11 nmol/L, $p = .046$). There was no difference between the groups regarding the daily insulin requirement.

Conclusion: Sensor-augmented pump therapy from onset of T1D may lead to better long-term glycaemic control and help to preserve endogenous beta-cell function, if patients comply with frequent use of continuous glucose monitoring.

Clinical Trial Registration Number: ISRCTN05450731

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Health-related quality of life among 11- to 17-year olds with early onset type 1 diabetes compared to peers of the representative German health survey KiGGS

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Background and aims: Health-related quality of life (HRQoL) is a potentially relevant determinant of the long-term course of type 1 diabetes (T1DM) among children and adolescents with early onset. Despite the increasing incidence of T1DM among children younger than 5 years, a population-based study on psychosocial factors in this patient-group was not available for Germany until now. The presented analyses aimed to compare HRQoL of 11- to 17-year olds with early onset of T1DM and at least 10 years diabetes duration with representative, normative data for the population of children and adolescents in Germany. In addition, risk factors for poor HRQoL were analysed.

Materials and methods: Inclusion criteria for the nationwide questionnaire survey conducted in Germany 2009–2011 were onset of T1DM in the age 0–4 years during 1993–1999. HRQoL was assessed by means of the generic 24-item KINDL-R self-report questionnaire (scale 0–100, higher values indicate better HRQoL). Representative, normative data came from the first KiGGS-study conducted in Germany 2003–2006. Analyses were performed with regression models (SAS 9.2, SURVEYREG procedure) including the parameters age, sex, migration background, region, socio-economic status, family arrangement, kind of school, BMI, hospitalisation and HbA_{1c} concentration (stated values mean (SD)).

Results: Survey participants were 629 11- to 17-year olds with T1DM (54% boys, age 15.3 (1.66) years, diabetes duration 12.5 (1.6) years, HbA_{1c} 8.3 (1.4) %). The reference group consisted of 6813 peers (51% boys, age 14.6 (2.0) years, HbA_{1c} 4.9 (0.4) %). Total HRQoL did not significantly differ between children and adolescents with T1DM (73.1 (12.2)) and the reference group (72.6 (10.3), $p = 0.092$) after adjustment for confounders. The sub-scale scores for physical and emotional well-being and friends did not significantly differ between the two groups. Well-being in the family was decreased (78.6 (18.2) versus 81.9 (15.7), $p = 0.003$), but both functioning at school (69.5 (17.8) versus 66.1 (17.2), $p < 0.001$) and self-esteem (63.3 (18.9) versus 58.3 (18.4), $p < 0.001$) were significantly increased among children and adolescents with T1DM. In the T1DM cohort, higher HbA_{1c} was associated with lower total HRQoL (change in HRQoL per 1% increase in HbA_{1c}: -1.8 , $p < 0.001$) and lower scores in the dimensions physical well-being (-2.1 , $p = 0.001$), emotional well-being (-1.0 , $p = 0.039$), self-esteem (-2.3 , $p < 0.001$), family (-2.8 , $p < 0.001$), and school (-3.2 , $p < 0.001$). Being adolescent (14–17 years) was associated with lower scores in the dimensions family (-3.9 , $p = 0.016$) and school (-4.6 , $p = 0.004$). Boys reported compared to girls higher total HRQoL (2.8 , $p = 0.004$), physical well-being (7.8 , $p < 0.001$), and self-esteem (6.5 , $p < 0.001$).

Conclusion: HRQoL among 11- to 17-year olds with early onset and long-lasting T1DM is not worse than among peers. In fact, T1DM is a potential resource for improved self-esteem in this patient-group (especially among boys). The results further underline the close relationship between metabolic control and HRQoL among children and adolescents with T1DM. Age and gender influence the HRQoL to some extent.

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OP 15 Gestational diabetes mellitus

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Gestational diabetes identified by the IADPSG- and WHO-criteria in a multiethnic population in Oslo, Norway: the Stork Groruddalen Research Program

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Background and aims: New criteria for gestational diabetes (GDM) with glucose threshold levels set to avert adverse fetal outcomes, has been suggested by the International Association of Diabetes and Pregnancy Study Group (IADPSG). The new criteria differ from the previous WHO criteria, mainly based on glucose threshold levels to identify women at risk of future diabetes. The aim of this study was to compare the prevalence rates and disparity in risk factors for GDM identified by the two criteria.

Materials and methods: Population-based cohort study of 823 (74% of the invited) healthy pregnant women attending three primary antenatal clinics in Eastern Oslo. 18 had abortion/preterm delivery, 33 drop-outs and 13 did not complete OGTT, leaving 759 with OGTT data. Fasting blood samples, anthropometrics and demographics were collected at visit 1 (V1), gestational week (GW) 15±5 and visit 2 (V2), GW 28±2. A 75-g OGTT was performed at V2, with venous blood samples analyzed on site (HemoCue, Angelholm, plasma calibrated). GDM by WHO: fasting plasma glucose (FPG) ≥7.0 mmol/l or 2-h PG ≥7.8 mmol/l, and IADPSG: FPG ≥5.1 mmol/l or 2-h PG ≥8.5 mmol/l. Insulin resistance was calculated using the homeostasis model assessment (HOMA2). Ethnic origin: Scandinavia (includes Western Europe, USA), Eastern Europe, South Asia, East Asia, Middle East, Somalia and Others. Simple descriptive, ANOVA (Bonferroni corrected), chi-square tests and logistic regression analyses were performed using PASW 18.

Result: GDM prevalence rates were 13% (WHO) and 32% (IADPSG). In total 9% were identified by both, 4% by only the WHO-criteria and 22% by only the IADPSG-criteria. Compared to Scandinavians all ethnic minority groups were shorter and had lower education ($p < 0.02$), South Asians were younger ($p < 0.001$) and together with Middle Easterners less likely to be para 0 and more likely to have 1° relatives with diabetes ($p < 0.03$), South Asians, Middle Easterners and Others were more insulin resistant at V1 ($p < 0.001$). GDM by the WHO-criteria was associated with age (OR=1.1, 95% CI: 1.1-1.2), parity ≥1 (OR 2.4, 95% CI: 1.4-4.0), 1° relatives with diabetes (OR 2.3, 95% CI: 1.4-3.8) and body height (cm) (OR 0.9, 95% CI: 0.88-0.96) after adjusting for ethnicity, age, BMI, body height, parity, education level and 1° relatives with diabetes. Adjusted for the same factors, GDM by the IADPSG-criteria was associated with prepregnant BMI (OR 1.1, 95% CI: 1.05-1.13) and South Asian origin (OR 2.5, 95% CI: 1.5-4.0).

Conclusion: The GDM prevalence by the WHO-criteria was high in all groups. The GDM prevalence increased 240% when applying the IADPSG-criteria. Before endorsing the IADPSG-criteria, the impact of diagnosing nearly 1/3 of all pregnant women should be considered.

Study sample divided into ethnic origin							
	Scandi- navia	Eastern Europe	South Asia	East Asia	Middle East	Somalia	Others
WHO GDM %	11	17	15	15	17	14	0
IADPSG GDM %	24	24	42	26	37	37	37
Years of age	31 (4.5)	29 (4.1)	29 (4.5)	31 (4.6)	29 (5.4)	29 (5.8)	29 (5.8)
BMI	24.6 (4.8)	23.8 (4.4)	23.7 (4.1)	22.3 (3.4)	25.9 (5.1)	26.8 (6.5)	26.3 (5.8)
prepregnant (kg/m2)							
Body height (cm)	167 (5.6)	166 (5.8)	160 (5.6)	157 (6.1)	161 (5.5)	164 (5.8)	162 (6.3)
Para 0, %	52	64	42	41	34	37	34
1° relatives with diabetes %	13	20	47	16	39	20	13
<10 years schooling %	3	12	18	21	38	60	13
FPG, V1 (mmol/l)	4.4 (0.4)	4.4 (0.4)	4.5 (0.4)	4.3 (0.3)	4.5 (0.5)	4.4 (0.5)	4.3 (0.5)
HOMA2-IR, V1 median (IQR)	0.7 (0.5)	0.7 (0.6)	1.1 (0.8)	0.8 (0.6)	0.8 (0.8)	0.8 (1.1)	1.0 (0.8)

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Gestational diabetes mellitus is a risk factor for future cardiovascular disease

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Background and aims: Women with gestational diabetes mellitus (GDM) have an increased risk of developing type 2 diabetes mellitus later in life. Less is known about whether these women also have an increased cardiovascular disease risk. The aim of this study was to examine GDM as a risk for subsequent cardiovascular morbidity and mortality using Swedish register data.

Materials and methods: A case-control study using the data from Swedish National Health and quality registers from 1991 to 2008. Cases defined as a woman diagnosed with a first cardiovascular event (Ischemic heart disease, stroke or peripheral vascular disease using the ICD classification) and who gave birth to at least one child during the study period ($n = 4653$). Each case was matched by age with 5 controls who did not have cardiovascular disease and also gave birth to a child during the study period ($n = 22790$). Binary logistic regression was used to calculate odds ratios with 95% confidence intervals (OR with 95% CI) with adjustment for potential confounding factors including smoking, chronic hypertensive disease and overweight (BMI>25).

Results: A history of GDM during pregnancy (3.5% among cases and 1.8% among controls) was associated with statistically significantly raised risk of a cardiovascular event, with unadjusted and adjusted odds ratios of 1.98 (1.64 - 2.37) and 1.80 (1.49-2.18), respectively. The unadjusted OR for overweight was 1.42 (1.33-1.52), for chronic hypertensive disease 4.86 (2.72-6.34) and for smoking 2.08 (1.95-2.22).

Conclusion: Women with a history of GDM not only have an increased risk of type 2 diabetes mellitus but GDM is also a notable risk for subsequent cardiovascular disease. This emphasizes the need for appropriate long-term follow-up of these women post pregnancy to identify their cardiovascular risk profile, possibly reducing their risk for cardiovascular events through appropriate interventions.

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Volatile organic compounds (VOCs) analysis in breath gas during 75g OGTT in women with suspected gestational diabetes mellitusJ.M. Maier¹, M. Hummel^{1,2}, S. Halbritter³, M. Fedrigo³, V. Höllriegel³, A.-G. Ziegler^{1,2}, W. Szymczak³,¹Forschergruppe Diabetes e.V., ²Institute of Diabetes Research, ³Department of Medical Radiation Physics and Diagnostics, Helmholtz Center Munich, Neuherberg, Germany.

Background and aims: Breath analysis in real time by using proton transfer reaction mass spectrometry (PTR-MS) is an innovative technology that can identify signatures and pathways that may be relevant for the pathogenesis of metabolic disorders. Furthermore, it can potentially be used as a non-invasive clinical diagnostic tool. In women with gestational diabetes mellitus (GDM), this technique has not been used and established yet. The aim of this study was to explore the applicability of the technology and the diagnostic potential of PTR-MS in woman suspect for GDM. We also describe metabolic products in expired air and assign them to metabolic pathways.

Materials and methods: We performed a 75g-OGTT in 53 consecutive pregnant women (median 28th gestational week). Parallel to OGTT we investigated in 6 minutes scanning the patient's exhaled air using the PTR-MS in real time. GDM was diagnosed in 8 patients (following the Carpenter criteria) and impaired glucose tolerance (IGT) in 13 patients. Thirty-two patients had normal glucose tolerance and formed the control group. Statistical analysis was performed by MANOVA and permutation analysis.

Results: We selected 17 volatile organic compounds (VOCs) in the exhaled air. Although there were fast and slow kinetics of the VOCs, all characteristic changes occurred within the first 45 minutes of the OGTT. Using the 17s VOC profiles we were able to accurately discriminate patients with GDM from patients with IGT, and from controls ($p=0.009$). Furthermore we were able to identify two distinct groups within the control group, one consisting of nine women, and one of the remaining 23 patients. Interestingly, the group of nine control women had significantly higher levels of fasting glucose (median 77 mg/dl vs. 71 mg/dl) and stimulated glucose (median 172 mg/dl vs. 147 mg/dl) than the remaining controls as well as higher triglyceride (TG) levels (median TG 211 mg/dl vs. 147 mg/dl). The correlation between VOCs profiles and metabolic status was a 'many masses', not a 'single mass' phenomenon. Nevertheless, two compounds could be identified which contributed most to the discrimination between patients and controls; these were related to the methionine and acetone metabolism.

Conclusion: Our data indicate that PTR-MS breath gas analyses are strongly correlated with glucose metabolisms during pregnancy and with diagnosis of gestational diabetes. Women with marginal values of glucose tolerance can be identified as distinct group that shows alterations of VOC signatures indicating pathology. Pathways utilizing methionine are potentially relevant for developing gestational diabetes.

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Osteocalcin- and ctx-associated hyperinsulinaemia rather than osteopontin-mediated insulin resistance play a key role in gestational diabetes mellitusY. Winhofer¹, F.W. Kiefer¹, A. Handisurya², A. Tura³, G. Pacini³, A. Luger¹, T.M. Stulnig¹, A. Kautzky-Willer¹;¹Department of Internal Medicine 3, Division of Endocrinology and Metabolism, Medical University of Vienna, ²Department of Internal Medicine 3, Division of Nephrology, Medical University of Vienna, Austria, ³Metabolic Unit, ISIB, CNR, Padova, Italy.

Background and aims: Reciprocal and regulatory interaction between bone and glucose metabolism might emerge early in the development of type 2 diabetes. Recently osteocalcin was found to enhance insulin secretion and insulin sensitivity in experimental animals. Furthermore, we could show increased serum concentrations of osteocalcin in women with gestational diabetes compared to women with normal glucose tolerance during pregnancy and a relation to hyperinsulinemia. The C-terminal cross-linking telopeptide of Type-I collagen (CTX, crosslaps) and osteopontin, two further hormones which were initially detected in bone metabolism, have recently been associated with glucose metabolism and obesity-induced inflammation and insulin resistance. However a distinct role in gestational diabetes, a model to study early changes in the development of type 2 diabetes, has not been investigated yet. Therefore we aimed to investigate serum concentrations of osteopontin

and CTX as well as parameters of insulin sensitivity and secretion in women with gestational diabetes and healthy controls.

Materials and methods: An oral glucose tolerance test for the assessment of insulin sensitivity and secretion as well as blood sampling for the measurement of CTX, osteopontin, high-sensitive CRP and serum lipids were performed between 24th and 28th gestational weeks in 26 women with gestational diabetes (GDM) and 52 women with normal glucose tolerance during pregnancy (NGT), well matched for age and BMI.

Results: CTX was significantly higher in GDM compared to NGT (0.44 ± 0.20 vs. 0.28 ± 0.12 , $p<.0001$) and positively correlated with parameters of insulin secretion (Total Insulin Secretion, AUC of insulin and C-Peptide, Disposition- and Adaptation Index), while it showed an inverse correlation with hepatic insulin extraction and HDL-cholesterol. Furthermore, CTX was significantly inversely correlated with insulin sensitivity derived from OGTT (OGIS: $R=-0.3$, $p=0.003$). In contrast, osteopontin was significantly decreased in GDM compared to NGT (1.15 ± 0.88 vs. 1.51 ± 0.79 ; $p<0.04$), and did not show any relation to insulin secretion or sensitivity, but was significantly correlated with high-sensitive CRP ($R=0.3$, $p<0.03$).

Conclusion: Our findings support the idea of a tight regulation between bone and glucose metabolism, and suggest, that osteocalcin- and CTX-associated hyperinsulinemia rather than osteopontin-mediated insulin resistance contribute to glucose intolerance in women with gestational diabetes. However, osteopontin may play a role in inflammatory changes (hsCRP) related to early disturbances of glucose metabolism.

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Maternal serum pregnancy associated plasma protein-A as predictor of gestational diabetes mellitusF. Jebunnesa¹, R. Karim², N. Sultana³, S. Hayat⁴, L. Ali¹;¹Dept of Biochemistry & Cell Biology, Bangladesh Institute of Health Sciences (BIHS), Dhaka, ²Dept of Biochemistry and Molecular Biology, Rajshahi University, ³Dept of Biochemistry & Cell Biology, BIRDEM, Dhaka, ⁴Dept of Gynecology and Obstetrics, BIRDEM, Dhaka, Bangladesh.

Background and aims: Pregnancy associated plasma protein-A (PAPP-A) has been shown to be a predictor for several pregnancy related complications including miscarriage, PIH and SGA. However, the predictive value of the protein has not been specifically investigated for gestational diabetes mellitus (GDM) which is a major complication of pregnancy and early prediction of which can largely contribute to the achievement of better maternal and fetal outcome. In the above context, we have tested the hypothesis that PAPP-A in early pregnancy can predict the development of GDM at the later stages.

Materials and methods: A group of 198 pregnant mothers, attending the OPD of our hospital, were enrolled in the study at 8-12 weeks of pregnancy and they were followed till delivery. A total of 102 (52%) women were found to develop GDM. From among the non-GDM cases a group of 96 women (the non-GDM group), matched for age and gestational age with the GDM group, were selected under a nested Case-Control design. GDM was diagnosed following the WHO Study Group Guidelines. Serum PAPP-A and serum insulin were measured by chemiluminescent ELISA techniques. Predictive value of early pregnancy PAPP-A in the development of GDM at later stages were explored by calculating the sensitivity, specificity, PPV and NPV using the McNamara test. Insulin secretory capacity (HOMA%B) and Insulin sensitivity (HOMA%S) were calculated by homeostatic model assessment and association of PAPP-A with GDM as well as with HOMA%B and HOMA%S were explored by univariate analysis and logistic /multiple regression as appropriate.

Results: PAPP-A (MOM) levels [median (range)] were markedly lower in GDM [$0.90(0.70-3.70)$] compared to the non-GDM [$6.40(2.6-10)$] groups ($p<0.001$). In Pearson's correlation analysis PAPP-A (MOM) had a significant positive correlation with HOMA%B. At optimum cut-off value of 5th percentile (2.6 mIU/m, calculated from an ROC curve) PAPP-A showed 99% sensitivity, 92% specificity, 93% PPV and 98% NPV in predicting the future development of GDM. On Logistic regression analysis PAPP-A showed significant association with insulin secretion ($p<0.001$) and insulin sensitivity ($p<0.001$) on adjusting the effects of the confounders.

Conclusion: Early pregnancy PAPP-A can be clinically useful in predicting the future development of GDM in later stages of gestation and it seems to have an association with insulin secretory defect and insulin resistance in this disorder.

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Vascular endothelial growth factor and brain derived neurotrophic factor in gestational diabetes mellitusJ.M. Brix¹, C. Hoebaus², E. Kratz¹, G.-H. Scherthaner², G. Scherthaner¹;¹Medicine I, Rudolfstiftung Hospital, ²Angiology, Medicine II, Medical University of Vienna, Austria.

Background and aims: Gestational diabetes mellitus (GDM) is a translational state of increased insulin resistance and accelerated atherosclerosis. Vascular endothelial growth factor (VEGF) and brain derived neurotrophic factor (BDNF) are potent mediators of angiogenesis and vasculogenesis. Therefore we were interested in VEGF and BDNF in patients with GDM compared to normal pregnancy (NGDM) and non-pregnant healthy controls (CO).

Materials and methods: We performed a cross-sectional and a longitudinal study, in which 45 patients with GDM (BMI 30.0 ± 5.3 kg/m², mean age 34 ± 5 years) as well as 16 pregnant women without GDM (NGDM, BMI 28.2 ± 4.4 kg/m², mean age 31 ± 5 years) were included and compared with 29 non-pregnant healthy controls (CO, BMI 24.1 ± 4.5 kg/m², mean age 32 ± 5 years). An oral glucose tolerance test (75g) and HOMA insulin resistance were assessed at 28 weeks of gestation as well as 8 weeks postpartum. Blood samples for VEGF and BDNF were obtained at the same time points and were determined by an ELISA.

Results: VEGF and BDNF levels were significantly lower in GDM patients compared to CO (1.6 ± 0.3 vs 2.5 ± 0.3 ; $p=0.013$; 22.1 ± 5.4 vs 26.4 ± 8.8 ng/ml; $p<0.001$). In addition, NGDM patients also had significantly lower VEGF and BDNF levels than CO (1.6 ± 0.2 vs 2.5 ± 0.3 ; $p=0.002$; 18.0 ± 6.9 vs 26.4 ± 8.8 ng/ml; $p<0.001$). Patients with GDM differed from NGDM only in BDNF significantly intrapartum (22.1 ± 5.4 vs 18.0 ± 6.9 ng/ml; $p=0.044$). We obtained a significant increase of VEGF and BDNF levels in GDM (2.6 ± 0.2 ; $p<0.001$; 27.7 ± 4.9 ng/ml; $p<0.001$) and NGDM (2.7 ± 0.1 ; $p<0.001$; 27.8 ± 0.8 ng/ml; $p=0.001$) patients postpartum. After pregnancy VEGF levels were significantly higher in the GDM group compared to CO (2.6 ± 0.2 vs 2.5 ± 0.3 ; $p=0.015$).

Conclusion: This is the first study demonstrating significantly lower VEGF and BDNF in pregnant women compared to healthy CO. These lower values of vascular growth factors may indicate that pregnancy per se is a protecting condition. BDNF and VEGF significantly increased postpartum in both GDM and non-GDM. The significant higher VEGF levels in GDM vs CO might be a contributing factor to the well-known increased cardiovascular risk of those patients.

OP 16 Nephropathy: experimental

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Glyoxalase-I overexpression attenuates early renal impairment in diabetic ratsO. Brouwers¹, P.M.G. Niessen¹, T. Miyata², J. Østergaard³, A. Flyvbjerg³, C.J. Peutz-Kootstra⁴, M. Brownlee⁵, C.D.A. Stehouwer¹, C.G. Schalkwijk¹;¹Department of Internal Medicine, Maastricht University, Netherlands,²Centre of Translational and Advanced Research, Tohoku University, Sendai, Japan,³Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Denmark,⁴Department of Pathology, Maastricht University Medical Center, Netherlands, ⁵International Center for Diabetic Complications Research, Albert Einstein College of Medicine, New York, USA.

Background and aims: In diabetes, intracellular glycation and elevated levels of advanced glycation endproducts (AGE) and AGE-precursor methylglyoxal (MGO) are associated with development of microvascular complications. In this study we used rats transgenically overexpressing the MGO-detoxifying enzyme glyoxalase-I (GLO-I) to determine the impact of intracellular glycation on the development of nephropathy in diabetes.

Materials and methods: Wild-type ($n=13$) and GLO-I transgenic rats ($n=18$) were made diabetic by streptozotocin (STZ) injection, sacrificed after 12 or 24 weeks and compared with controls ($n=14$). The levels of the AGEs, N^ε-(1-carboxymethyl)lysine (CML) and N^ε-(5-hydro-5-methyl-4-imidazolonyl)-L-ornithine (MG-H1) were measured with tandem mass-spectrometry and immunohistochemical stainings. Kidneys were isolated, weighted and analyzed for structural alterations, collagen content and glomerular volume. The early urinary nephropathy markers, albumin, osteopontin, lipocalin-2 and kidney injury molecule-1 (KIM-1), were measured by ELISA-based techniques.

Results: Diabetes resulted in accumulation of plasma AGEs, which was decreased by overexpression of GLO-I. In addition, immunohistochemical analysis showed increased presence of CML and MG-H1 in the mesangial matrix and peritubular capillaries of the diabetic rats, which could not be prevented by GLO-I overexpression. Diabetes resulted in renomegaly and increased glomerular volume after both timepoints, which were decreased, but not significantly improved by GLO-I overexpression. Histological analysis of the kidney did not show any collagen deposition fibrotic or inflammatory differences in glomerular, tubulointerstitial or capillary tissue between the three groups after 24 weeks of diabetes. After 24 weeks of diabetes, creatinine clearance was elevated, without improvement by GLO-I overexpression. Early urinary nephropathy markers, i.e. albumin, osteopontin, lipocalin-2 and KIM-1, were increased after 12 weeks of diabetes and further increased after 24 weeks of diabetes. The increase of albumin, osteopontin and KIM-1 after 24 weeks of diabetes was significantly attenuated by GLO-I overexpression. Finally, these biomarkers of nephropathy strongly correlated with plasma AGE levels.

Conclusion: Overexpression of GLO-I attenuates the development of early diabetic nephropathy, which is not dependent on structural changes of the kidney, suggesting effects due to an improvement in vascular/endothelial function. Inhibition of intracellular glycation by activation of GLO-I or quenching of MGO may provide a novel approach to prevent diabetic nephropathy.

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SDF-1/CXCR4-mediated crosstalk attenuates albuminuria in experimental diabetic nephropathyL.-H. Chen¹, S.L. Advani¹, K. Thai¹, M.M. Sood², I.W. Gibson²,K.A. Connelly¹, R.E. Gilbert¹, A. Advani¹;¹Keenan Research Centre of the Li Ka Shing Knowledge Institute, St.Michael's Hospital, Toronto, ²Health Sciences Centre, University of Manitoba, Winnipeg, Canada.

Background and aims: The stromal cell-derived factor-1 (SDF-1)/CXCR4 signaling pathway exemplifies a podocyte-endothelial crosstalk system essential for renal vascular development. Within the renal glomerulus, while the homeostatic chemokine SDF-1 is abundantly expressed by podocytes, its cognate receptor, CXCR4, is present on the surface of endothelial cells where it may signal through the PI3K/Akt/eNOS pathway. In the context of recent evidence indicating that high glucose concentrations directly augment the

activity of hypoxia inducible factor-1 α (HIF-1 α), the major upstream regulator of CXCR4 expression, we investigated the role of SDF-1/CXCR4 signaling in human and experimental diabetic nephropathy.

Materials and methods: Gene and protein expression were determined in rat and human kidneys. CXCR4-antagonism was achieved by administering the bicyclam, AMD3100, to streptozotocin-diabetic rats and eNOS knockout mice.

Results: Immunostaining rat kidney sections confirmed podocytic expression of SDF-1, with CXCR4 protein restricted to endothelial cells of glomerular and peritubular capillaries. Real-time PCR of kidney homogenates from rats after 4 weeks of streptozotocin-induced diabetes revealed upregulation of both HIF-1 α and CXCR4 mRNA (HIF-1 α (AU), control 1.04 ± 0.09 , diabetes 1.68 ± 0.11 ; CXCR4 (AU), control 1.00 ± 0.04 , diabetes 1.70 ± 0.14 , each $p < 0.001$). CXCR4 mRNA was similarly increased in biopsies from patients with diabetic nephropathy (DN) relative to time-zero live kidney donor tissue (control) (CXCR4 (AU), control 1.09 ± 0.16 , DN 4.14 ± 0.79 , $p < 0.01$). Treatment of diabetic rats with the CXCR4 antagonist, AMD3100, augmented albuminuria (albumin excretion rate (AER) ($\mu\text{g}/24\text{h}$), vehicle 177.8 ± 1.2 , AMD3100 331.1 ± 1.2 , $p < 0.05$) without affecting body weight, blood glucose or blood pressure. In contrast, AMD3100-treatment had no effect on the heavy albuminuria that occurred in streptozotocin-diabetic eNOS knockout mice (AER ($\mu\text{g}/24\text{h}$), vehicle 302.0 ± 1.2 , AMD3100 281.8 ± 1.2 , $p = 0.81$).

Conclusion: These observations indicate that high-glucose mediated upregulation of CXCR4 serves to attenuate albuminuria development in diabetic nephropathy. Since podocytes are the primary source of CXCR4-ligand (SDF-1) within the renal glomerulus and since the albuminuric effects of CXCR4-blockade are abrogated by deficiency of an ostensibly endothelial-specific gene (eNOS), the SDF-1/CXCR4 signaling pathway defines a podocyte-endothelial crosstalk system that is altered in diabetic nephropathy. Therapeutic augmentation of CXCR4-signaling may represent a novel therapeutic strategy to slow the progression of renal injury in diabetes.

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Effect of CB2 receptor deletion in streptozotocin-induced diabetic mice

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Background and aim: Diabetic nephropathy (DN) is characterised by increased glomerular permeability to proteins and excessive extracellular matrix accumulation in the mesangium, resulting eventually in glomerulosclerosis and progressive renal impairment. Endocannabinoids (EC), anandamide and 2-arachidonoylglycerol, bind to two receptors, named CB1 and CB2. In experimental diabetes the CB1 is overexpressed by podocytes and CB1 blockade ameliorates albuminuria. The CB2 is predominantly expressed by immune cells; however, we have previously reported preliminary data showing that the CB2 is also present on glomerular podocytes and that a CB2 agonist reduces albuminuria in diabetic mice. To further explore the role of CB2 in DN, herein we studied functional and structural abnormalities of DN in diabetic mice knockout for the CB2 receptor.

Materials and methods: Both wild type (CB2^{+/+}) and CB2 knockout (CB2^{-/-}) mice were made diabetic by intraperitoneal (IP) injection of streptozotocin at a dose of 55 mg/kg delivered in 3 consecutive days. Control mice were injected with citrate buffer alone. 16 weeks after the induction of diabetes, mice were individually placed in metabolic cages for urine collections and blood samples taken for blood glucose and glycated haemoglobin measurements. Then, mice were sacrificed, kidneys removed, weighed, and analysed. Urinary albumin excretion was measured by enzyme-linked immunosorbent assay. Expression of slit-diaphragm proteins (nephrin and podocin) was assessed by both immunofluorescence and immunoblotting. Fibronectin, CTGF, collagen I and TGF- β 1 mRNA levels were quantitated by real-time PCR on total renal cortex. Markers of inflammation (MCP-1, CCR2, F4/80 and GR1) were analysed by real time PCR or immunohistochemistry. Histological damage was assessed by both PAS staining and electron microscopy.

Results: Diabetes was associated with reduced body weight and elevations in both plasma glucose and glycated haemoglobin levels, but no differences were seen between CB2^{+/+} and CB2^{-/-} mice. Albuminuria was significantly increased in the wild type diabetic animals as compared to the controls and further enhanced by CB2 receptor deletion. In the diabetic mice the increase in albuminuria was paralleled a significant reduction in both nephrin and podocin protein expression and this effect was further exacerbated in diabetic mice lacking CB2 receptors. Histological assessment by PAS staining showed that the degree of mesangial expansion was greater in diabetic mice CB2^{-/-}

than in wild type mice as confirmed by electron microscopy. Consistently, diabetes-induced overexpression of fibronectin, CTGF, collagen I, and TGF- β 1 mRNA levels was significantly increased by CB2 deletion. Finally, lack of CB2 receptor induced a strong CCR2 upregulation in both control and diabetic mice and significantly enhanced monocyte infiltration in diabetic mice.

Conclusion: These findings demonstrate that in experimental diabetes CB2 deletion worsens both functional and structural abnormalities of DN. This strengthens the argument of a protective role of signalling through the CB2 receptor in this complication.

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Effects of intrarenal tonic activation of angiotensin II and adenosine receptors on renal haemodynamics of control and diabetic rats

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Background and aims: Diabetic nephropathy (DN) is characterized by enhanced renal renin-angiotensin system (RAS) activity and renal vascular dysfunction. Both angiotensin II and adenosine influence renal vascular tone. Adenosine restrains renin release from juxtaglomerular cells and therefore may be an important modulator in DN. This study aimed at evaluating the effects of *in vivo* intrarenal blockade of angiotensin II AT₁ and AT₂ receptors and adenosine A₁ and A₂ receptors on renal hemodynamic in a model of DN.

Materials and methods: On day 14 after diabetes induction, streptozotocin-induced (50 mg/kg; iv; diabetic) or vehicle (controls) male Sprague-Dawley rats (≈ 340 g) were anaesthetized and prepared for surgery. The left kidney was immobilized and a lumbar catheter was advanced about 1 mm into the left renal artery to allow kidney specific delivery of vasoactive substances. The angiotensin II AT₁ receptor antagonist candesartan ($4.2 \mu\text{g}/\text{kg}$) and the adenosine A₁ receptor antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX; $140 \mu\text{g}/\text{kg}$) were infused in the renal artery after a baseline period, in separate groups. An additional group received the combination of candesartan and DPCPX. The infusion was made in a step wise manner during 10 min to minimize leakage to the systemic circulation. In some animals, an additional experimental period was done subsequent to the infusion of angiotensin II AT₁-receptor antagonist PD-123,319 ($0.5 \text{ mg}/\text{kg}$) or adenosine A₂-receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX; $0.5 \text{ mg}/\text{kg}$). GFR was measured by ³H-inulin clearance and renal blood flow (RBF) was measured by a transonic flow probe placed around the left renal artery. Statistical differences were evaluated by ANOVA followed by Tukey's test for multiple comparisons or Student's paired *t*-test, as appropriated.

Results: Baseline RBF was similar in all groups, averaging $8 \pm 0.1 \text{ ml}/\text{min}/\text{kidney}$. Intrarenal treatment of both diabetic and control animals with candesartan, DPCPX or candesartan+DPCPX increased RBF in approximately 30 % ($p < 0.05$). Baseline GFR and filtration fraction (FF) were higher in diabetic animals compared to controls (averaging 4 ± 0.2 vs $2 \pm 0.1 \text{ ml}/\text{min}$, 42 ± 2 vs $20 \pm 2 \%$, respectively, $p < 0.05$). In control animals, treatment with DPCPX or candesartan+DPCPX increased GFR when compared to baseline (1.8 ± 0.21 vs 1.5 ± 0.2 and 2.1 ± 0.3 vs $1.6 \pm 0.2 \text{ mL}/\text{min}$, $p < 0.05$, respectively). In diabetic animals, candesartan infusion reduced GFR (3.0 ± 0.3 vs $3.8 \pm 0.3 \text{ mL}/\text{min}$, $p < 0.05$) when compared to baseline. Baseline FF was reduced after the infusion of candesartan, DPCPX or candesartan+DPCPX in approximately 25 % ($p < 0.05$) in diabetic animals. Infusion of PD-123,319 after candesartan or DMPX after DPCPX, had no additional effect in these parameters.

Conclusion: Tonic activation of angiotensin II AT₁ and adenosine A₁ receptors in the afferent arteriole is the main regulator of renal vasoactivity in control animals. Interestingly, in diabetic animals the efferent arteriole seems to have a pivotal role. Our study also suggests that tonic activation of angiotensin II AT₂ and adenosine A₂ receptor does not participate in the process.

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High NEFA level related with microalbuminuria and uncoupling of VEGF-NO axis in obese rats

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Background and aims: Obesity is associated with microalbuminuria (MAU), which is not only an early manifestation of kidney damage, but also an independent risk factor for ischemic cardiovascular disease. High NEFA level is a common feature of obesity and can cause impaired endothelium-dependent vasodilatation (EDV). It is known that the beneficial effects of vascular endothelial growth factor (VEGF) are mediated in part by ability to stimulate endothelial nitric oxide (NO) production, and thus maintain glomerular endothelial cells function. However, endothelial dysfunction may cause the uncoupling of VEGF with NO, resulting in increased levels of VEGF and excessive endothelial cell proliferation. The aim of this study was to determine whether increased NEFA release from excessive visceral fat accumulation is related to increased urine albumin excretion and reduced circulating NEFA level by fenofibrate has renal protective effects by improving endothelial dysfunction and regulating VEGF-NO axis in diet-induced obese rats.

Materials and methods: Male Wistar rats 8 weeks in age were randomly divided into three groups. The rats in the control group, obesity group and fenofibrate group were fed with normal diet (330 kcal 100 g⁻¹), high-fat diet (493 kcal 100 g⁻¹), and high-fat diet plus fenofibrate (100mg/kg/d), respectively. Blood samples for NEFA, triglyceride (TG) measurements and urinary samples for albumin measurements were collected. Endothelial function was determined by comparing the vasorelaxation response of thoracic aorta segment to acetylcholine (to determine EDV), with that of sodium nitroprusside (to determine endothelium-independent vasodilatation) in organ bath. Renal cortex tissues were collected for CD31 immunohistochemistry. Glomerular NO and VEGF expression were measured by griess reaction and western blot, respectively.

Results: At the end of 24 weeks, body weight increased 32.8% in obese rats compared to control group. The serum NEFA and TG levels increased significantly in obese rats (NEFA, 1.38±0.34 vs. 0.36±0.12; TG, 1.53±0.58 vs. 0.52±0.20, P<0.01). Fenofibrate intervention decreased serum NEFA and TG levels by 43.4% and 48% respectively, accompanied by reduced ratio of perirenal fat to body weight (PF/BW) and visceral fat to body weight (VF/BW). Urinary albumin/creatinine ratio (ACR) increased in obese rats (56.09±22.64 vs. 19.52±7.30 mg/g, P<0.01), which was decreased 62.6% after fenofibrate intervention. Severe EDV impairment was observed in obese rats, which was partly improved by fenofibrate (EDV increased 43.3%, P<0.05). CD31 expression in glomeruli increased in obese rats (P<0.05), indicating that increased endothelial cell proliferation. Obese rats showed increased glomerular VEGF expression (P<0.05) and reduced NO level (0.30±0.09 vs. 0.53±0.08 umol/g protein, P<0.05). And this uncoupling of VEGF-NO axis were partly improved by fenofibrate (P<0.05). Correlation analysis showed ACR had a positive correlation with NEFA, PF/BW and VF/BW (r=0.751, 0.783, 0.712, respectively, P<0.01) and had a negative correlation with EDV (r=-0.781, P<0.01).

Conclusion: Elevated circulating NEFA level may cause increased MAU in obese rats by impairment of EDV and increased MAU could be improved by reducing circulating NEFA level. The mechanism may be related to NEFA-induced uncoupling of VEGF-NO axis and the abnormal proliferation of endothelial cells.

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P2X₇ deficiency attenuates renal disease induced by high-fat dietA. Solini¹, S. Menini², C. Rossi¹, C. Iacobini², E. Santini¹, C. Blasetti Fantauzzi², A. Salvati¹, G. Pugliese²;¹Dept of Internal Medicine, University of Pisa, ²Dept of Clinical and Molecular Medicine, La Sapienza University, Rome, Italy.

Background and aims: Renal disease associated with type 2 diabetes and the metabolic syndrome is characterized by a distinct inflammatory phenotype. The purinergic system plays a relevant role in the inflammatory and immune response to injurious stimuli, particularly through the P2X₇ receptor, which is expressed in resident and non-resident kidney cells, such as mesangial cells and macrophages. In addition to stimulating the release of pro-inflammatory cytokines, it has been shown to promote mesangial cell apoptosis and ma-

trix production, these effects being opposed by the P2Y receptors, and to be upregulated in glomeruli from animal models of diabetes, hypertension and glomerulonephritis. This study was aimed at verifying the hypothesis that P2X₇ promotes the development of lipid-induced renal disease through the induction of renal tissue inflammation and consequent dysregulated glomerular remodeling.

Materials and methods: P2X₇ knockout (KO) mice and coeval wild type (WT) controls were fed a high-fat diet (HFD, 60% saturated fat) or a normal-fat diet (NFD, 4% saturated fat) for 4 months. Glomerular lesions were evaluated by measuring mean glomerular area, mean mesangial area, and fractional mesangial area by morphometrical analysis. Glomerular protein expression and distribution and total kidney gene expression were assessed by immunohistochemistry and RT-PCR, respectively. Renal signalling events were evaluated by Western blot analysis.

Results: Body weights were significantly higher in KO vs. WT mice and increased in both genotypes upon HFD. HFD-induced glomerular lesions were attenuated in KO vs. WT mice. This was confirmed by morphometrical analysis, showing significantly lower values (P<0.001) in KO vs. WT mice of mean glomerular area (3,165±183 vs. 3,453±212 μm²), mean mesangial area (605.3±60.3 vs. 780.8±80.8 μm²), and fractional mesangial area (19.2±2.0 vs. 22.7±2.6 %). This was associated with significantly reduced glomerular expression of the extracellular matrix proteins fibronectin and collagen IV, the macrophage marker F4/80 and the advanced lipoxidation endproducts 4-hydroxy-2-nonenal (HNE)-adducts as well as with decreased kidney mRNA levels of collagen IV, F4/80 and the pro-inflammatory cytokine monocyte chemoattractant protein-1 (MCP-1). Phosphorylation of ERK1/2-mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) were up-regulated upon HFD in WT and KO mice, respectively.

Conclusion: These data show that P2X₇ plays an important role in HFD-induced renal disease by modulating glomerular cell apoptosis and matrix accumulation and renal tissue inflammation.

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OP 17 GWAS and next generation sequencing

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Low-pass whole-genome sequencing, deep exome sequencing and dense array genotyping for determining genetic variants associated with type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) is a complex disorder of incompletely known genetic etiology. Although lower-frequency and rare variants likely contribute to T2D susceptibility, these variants have not yet been thoroughly investigated on a large scale. In addition, association between small insertions and deletions (indels) and T2D has not previously been assessed on a genome-wide scale. In this study, we are attempting to identify additional variants contributing to T2D susceptibility in 1,325 T2D cases and 1,325 controls of Finnish, Swedish, and UK origin.

Materials and methods: We are using several approaches to target lower frequency and indel variants, including low-pass (4x) whole-genome sequencing, deep exome sequencing, array genotyping of 2.5 million SNPs, and genotype imputation using sequence data generated both within this project and from the 1000 Genomes project. Analysis of data from each approach, both independently and jointly, will help elucidate the genetic architecture of T2D across a wider allele frequency spectrum of both SNPs and indels, as well as the most effective approaches to achieve this end.

Results: Preliminary analysis of data from a first-term data freeze representing 179 low-pass genomes identified 11.3 million SNPs, 40% of which are not present in public databases. Among novel, rare variants are several unique to cases that create or delete a stop codon (nonsense) in genes at loci involved in monogenic (PAX4-W91*, WFS1-E752*) and polygenic (TSPAN8-*238R) forms of T2D. In addition, several novel nonsense variants unique to cases implicate previously unreported genes (e.g. GPR37-R68*); case allele frequency = 5%, uncorrected $P = .025$). Variants were tested for T2D association after imputation into 4,100 case and 5,200 control samples with genome-wide array data from the DGI, FUSION and WTCCC studies. Patterns of association in common variants were consistent with previous results imputing HapMap data into the same samples, including three established T2D loci with $P < 5 \times 10^{-8}$, suggesting that variants identified in low-pass sequencing can be effectively tested in larger sample sets to maximize risk allele discovery potential.

Conclusion: Data collection is ongoing with all data generation expected to be complete by autumn 2011, which will greatly enhance statistical power for discovery both directly and via imputation. This project represents the first opportunity to gain broad insight into the contribution to type 2 diabetes of different types of variant across the allele frequency spectrum.

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Genome-wide association analysis of rare variants with type 2 diabetes

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Background and aims: Genome-wide association studies (GWAS) of common variants have been extremely successful in identifying novel loci contributing effects to type 2 diabetes (T2D) susceptibility. However, despite this success, the joint effects of these common variants account for no more than 15% of the heritability of the disease. The aim of this study, therefore, was to assess the evidence for association of T2D with rare genetic variation, defined here to have minor allele frequency (MAF) less than 1%, which is typically poorly captured by GWAS genotyping products.

Materials and methods: We performed imputation in 1,926 T2D cases and 2,942 controls of European descent from the UK, genotyped using the Affymetrix GeneChip 500K Mapping Array Set as part of the Wellcome Trust

Case Control Consortium. Imputation was undertaken using IMPUTEv2 and the European reference panel from the August 2010 release of the 1000 Genomes Project. We tested for association of T2D with accumulations of minor alleles at rare variants (imputed and directly typed) within genes, incorporating 50kb up- and down-stream to allow for additional functional elements and regulatory regions. The analysis was performed using GRANVIL, which models disease status as a function of the proportion of rare variants at which an individual carries at least one minor allele in a logistic regression framework.

Results: The strongest signal of association of rare variation with T2D was observed for BMP2 ($p = 1.0 \times 10^{-6}$, genome-wide significant correcting for 30,000 genes). Common variants within this gene have previously been demonstrated to be associated with developmental and metabolic traits including height and body mass index. The gene contained 36 rare variants (mean MAF = 4.3×10^{-3}), with odds ratio of 1.26 (1.15–1.38) per minor allele. Strong evidence of association of rare variation with T2D ($p < 10^{-3}$) was also observed for IGFL4 ($p = 2.4 \times 10^{-6}$) and CLK3 ($p = 6.4 \times 10^{-6}$). IGFL4 belongs to a family of signalling molecules that play crucial roles in cellular energy metabolism and in growth and development. The gene contained 12 rare variants (mean MAF = 4.4×10^{-3}), with odds ratio of 1.85 (1.43–2.38) per minor allele. CLK3 contained 22 rare variants (mean MAF = 3.1×10^{-3}), with odds ratio 0.65 (0.54–0.79) per minor allele, hence demonstrating a protective effect against T2D.

Conclusion: Our analysis has demonstrated strong evidence of association of T2D with rare variation in three genes. These signals of association warrant follow-up in independent cohorts, and parallel efforts are underway in the DIAGRAM Consortium. Our results highlight the potential for the identification of rare variant associations using existing GWAS genotyping data, supplemented with imputation from high-density reference panels, without the need for costly re-sequencing experiments.

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Large-scale replication using “MetaboChip” array identifies additional genetic loci influencing glycaemic traits

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Background and aims: The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) has previously performed the largest published meta-analysis of genome-wide association studies (GWAS) for glycaemic traits to date, bringing the number of established loci for fasting glucose (FG) to 16 and fasting insulin (FI) to 2. The “MetaboChip”, a custom iSELECT array of 217,697 SNPs, was developed to support large-scale follow-up of putative associations for cardio-metabolic traits and fine-mapping of established loci. It contains >65,000 top-ranked, independent SNPs selected from the stage 1 MAGIC discovery meta-analyses (up to 46,186 non-diabetic Europeans), including ~5k/1k selected for FG/FI ($10^{-75} < P < 0.02$).

Materials and methods: To identify additional loci for FG/FI, we combined SNPs selected for replication in the MetaboChip in 24/21 independent cohorts (53,149/42,252 individuals) with MetaboChip data with the non-overlapping MAGIC GWAS discovery cohorts and an additional 15/13 GWAS (25,618/23,130 individuals) using a fixed effects meta-analysis. The combined meta-analysis included a total of up to 120,845/99,610 individuals. Association analyses in each cohort were performed using an additive genetic model.

Results: We identified 15 novel genome-wide significant ($P < 5 \times 10^{-8}$) loci for FG, nine of which have been previously associated with other metabolic traits: SNPs in or near CDKN2A/2B, FOXA2, GRB10 (type 2 diabetes [T2D]), CENTD2/ARAP1 (T2D, proinsulin), PDX1 (MODY), PCSK1 (obesity, proinsulin), GIPR (2h glucose), LIN28B (age at menarche, height) and PPP1R3B (LDL-cholesterol and C-reactive protein). The remaining 6 novel loci were located in or near genes encoding for transcriptional repressors (14q31, 20q12), tRNA synthetase (14q32), nucleotide phosphodiesterase (10q24), purinoreceptors (12q24) and proinflammatory signaling kinase (9q31). We discovered 4 novel loci for FI at a genome-wide level of significance, including variants in or near FTO (BMI), TCF7L2 (T2D, FG, HbA1C), PPP1R3B and at 4q24 (in gene encoding for myelopoiesis-related protein). A further 7 loci attained “suggestive” evidence of association ($P < 5 \times 10^{-7}$) with FI. These loci

included LYPLAL1 (WHR, fat distribution, fatty liver), CENTD2/ARAP1, ARL15 (T2D, adiponectin).

Conclusion: Replication using the MetaboChip array has allowed us to extend the number of genetic loci for FG to 31. For FI, only 6 genome-wide significant and another 7 loci just below have been identified, a number that remains small compared to other traits. Identification of FI loci previously associated with lipids and fat distribution highlights a potential link to hepatic insulin resistance. The overlap between the novel FG/FI loci and established loci for T2D, metabolic and anthropometric traits reinforces the complex relationships that exist between these phenotypes. The MetaboChip provides an efficient opportunity to widen our knowledge about the genetics of fasting glycaemic traits.

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Genome-wide association meta-analysis identifies novel genetic loci for birth weight in Europeans

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Background and aims: Reduced birth weight (BW) is associated with a higher risk of type 2 diabetes (T2D), but the mechanisms responsible are unclear. The fetal insulin hypothesis proposes that common genetic variants influencing insulin secretion or action explain part of this association because fetal insulin is a key fetal growth factor. BW may be influenced directly by fetal genotype and indirectly by maternal genotype through the intra-uterine environment and interaction of the fetal-maternal genotypes. Despite these challenging aspects involved in identification of BW susceptibility genes, previous genome-wide association (GWA) meta-analysis successfully identified variants in ADCY5 and near CCNL1 robustly associated with BW. Here we extend this effort by increasing the discovery sample.

Materials and methods: The discovery GWA meta-analysis of BW was performed in 18 studies (26,864 individuals) and one SNP at each of 17 associated loci ($p < 10^{-5}$) was selected for follow up in replication studies. We genotyped these variants in up to 37,120 participants from 18 cohorts of European descent. For analysis we considered singletons born at ≥ 37 weeks' gestation. We tested the association of each SNP using standardized Z-score of birth weight estimated in each study, assuming an additive genetic model and adjusting for sex and gestational age where available. We combined the results from replication and discovery studies (up to 63,941 individuals) using inverse-variance fixed-effects meta-analysis. We used a threshold of $p < 5 \times 10^{-8}$ to define the loci as genome-wide significant.

Results: SNPs in six loci were associated with BW at $p < 5 \times 10^{-8}$ or close to this threshold. The first two loci map near the CCNL1 gene ($p = 2.9 \times 10^{-28}$) and in ADCY5 ($p = 3.5 \times 10^{-15}$), which were both identified in the previous study. The next two identified signals map, respectively, to chromosome 12q15 ($p = 2.9 \times 10^{-19}$) and to the known susceptibility locus for T2D at CDKAL1 ($p = 8.8 \times 10^{-17}$). Two more independent signals on chromosome 4 were associated with BW. The former maps to chromosome 4p15 ($p = 1.1 \times 10^{-8}$) and the latter to chromosome 4q28 ($p = 8.8 \times 10^{-8}$). Of the six BW loci, three signals (12q15, 4p15 and 4q28) were in LD with known loci influencing adult height.

Conclusion: In our replication of expanded GWAS meta-analysis for birth weight, we confirmed 2 previously identified signals (in ADCY5 and near CCNL1) and discovered 4 novel BW genome-wide significant loci. These 6 signals include 3 known loci influencing adult height and 2 known T2D susceptibility loci (ADCY5 and CDKAL1). The alleles at the loci associated with an increased risk of T2D were also associated with lower BW. This is consistent with the fetal insulin hypothesis, which proposes that common genetic variants influencing insulin secretion play a key role in both low BW and later life chronic diseases. Identification of signals in LD with adult height loci suggests the impact of these loci in both pre- and post-natal growth.

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Identification of novel type 2 diabetes susceptibility loci by large-scale replication using the “MetaboChip”

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Background and aims: There are now more than 40 established loci influencing susceptibility to type 2 diabetes (T2D), most identified through genome-wide association studies. However, replication efforts have focussed on only the strongest signals of association, and have failed to fully exploit large-scale discovery meta-analyses. The “MetaboChip”, a custom iSELECT array containing ~195,000 SNPs, was designed to support large-scale follow-up of putative associations for T2D and other metabolic and cardiovascular traits, as well as fine-mapping of established loci.

Materials and methods: We performed meta-analysis of MetaboChip SNPs for 21,556 cases and 55,753 controls across 25 cohorts of European descent and 1,178 cases and 2,472 controls from a cohort of Pakistani descent. We subsequently combined these results with the unpublished DIAGRAMv3 meta-analysis (12,057 cases and 56,071 controls of European descent). Association analyses in each cohort were performed assuming additive allelic effects and the results combined via fixed-effects meta-analysis. These analyses were undertaken in males and females separately, and in both sexes combined.

Results: We began by considering 4,747 high quality, statistically independent SNPs which capture $>95\%$ of the ~5,000 strongest autosomal associations from the DIAGRAMv3 meta-analysis. At these SNPs, we compared patterns of replication between the DIAGRAMv3 and European descent MetaboChip sex-combined meta-analyses. After excluding SNPs at established loci, there was highly significant concordance in the direction of allelic effects (3,066 [65.3%] of 4,696 SNPs, binomial test $p = 8.8 \times 10^{-94}$). As expected, the directional consistency with the Pakistani descent cohort was weaker, but still highly significant (2,320 [53.3%] of 4,352 SNPs, binomial test $p = 6.8 \times 10^{-6}$). Combined meta-analysis of all samples at all MetaboChip SNPs revealed 11 novel loci exceeding genome-wide significance, the strongest signals mapping to ZMIZ1 ($p = 7.8 \times 10^{-11}$), GOLGA7 ($p = 1.6 \times 10^{-10}$) and PPFIBP1 ($p = 3.5 \times 10^{-10}$). Sex-differentiated meta-analyses highlighted a further 4 novel loci exceeding genome-wide significance, the strongest signal mapping to CCND2 ($p = 5.9 \times 10^{-10}$) where the association was markedly stronger in males ($p = 7.7 \times 10^{-10}$) than in females ($p = 0.032$). There was also evidence of heterogeneity in allelic effects between the sexes in already established T2D loci mapping to KCNQ1 (male $p = 6.8 \times 10^{-15}$, female $p = 0.0060$), DGKB (male $p = 4.6 \times 10^{-13}$, female $p = 0.0042$), and BCL11A (male $p = 1.1 \times 10^{-9}$, female $p = 0.013$).

Conclusion: The concordance of risk alleles between DIAGRAMv3 and MetaboChip meta-analyses, both within and between ethnic groups, is consistent with a long tail of common variants with modest effect on T2D susceptibility across populations. Further MetaboChip genotyping thus offers great promise for further discovery of T2D loci through inter- and intra-ethnic mapping studies, whilst sex-differentiated analyses may provide additional insights into the underlying genetic architecture of the disease.

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Selection of extreme cases and controls as a strategy for efficient whole genome sequencing leads to enriched detection of most known type 2 diabetes susceptibility loci

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Background and aims: Sequencing-based studies allow exploration of the role of low frequency variants in common disease susceptibility. However, given the current extremely high cost of whole genome and exome sequencing, such studies naturally have to focus on the most informative subjects. Type 2 diabetes (T2D) patients with positive family history and early age of onset are likely to be enriched for low frequency loci with medium penetrance: selection for relatively lean cases should further enrich for effects on beta-cell function. By analogy, the most informative controls are likely to be those who remain normoglycemic at advanced age, particularly in the face

of obesity. The Genetics of T2D (GoT2D) study adopted this sample selection strategy to perform low-pass whole genome, deep exome sequencing and high-density array genotyping of 2650 individuals from the UK, Sweden and Finland.

Materials and methods: To establish whether these enrichment effects are visible with known (common) T2D-susceptibility loci, we examined the selected UK GoT2D samples. Cases ($n=337$) from the Warren 2 collection were selected for first degree family history of T2D, younger age of diagnosis (mean=50.4[SD=8.3] years) and lower BMI (26.5[2.6] kg/m²). Euglycemic controls ($n=351$) were chosen from the TwinsUK cohort to be older (61.4[10.0] years) with higher BMI (30.7[5.9] kg/m²) than cases. Cases and controls were also matched on principal components. These samples were genotyped on the 2.5M Illumina OmniChip (1,670,266 polymorphic SNPs passing QC). We compared signal effect size to those previously-reported by the DIAGRAM consortium using the ratio between the two effect size estimates.

Results: Among the studied loci, TCF7L2 (OR [95%CI], 2.1 [1.6-2.7]), DUSP9 (1.7 [1.2-2.5]), and ADCY5 (1.5 [1.2-2.0]) showed the largest effect sizes with the TCF7L2 association reaching genome-wide significance ($P=1.3 \times 10^{-9}$) despite the modest sample size. On average, the point estimate of per-allele risk was ~7% higher for the selected samples. Furthermore, 8 loci (DUSP9, TCF7L2, ADCY5, CDC123/CAMK1D, RBMS1, CENTD2, HHEX/IDE, GCK), all acting through presumed beta-cell effects, showed the most marked increases (20-58%).

Conclusion: Analysis of subjects selected from extremes of the liability distributions achieved the desired effect of enhancing effect size estimates for known common variants. Focusing sequencing efforts in such samples should aid discovery of novel low frequency associations.

OP 18 Signal transduction

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Hydrogen peroxide is a nutrient-activated secondary signal in beta cells

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Background and aims: The islet β -cell is thought to be susceptible to damaging reactive oxygen species (ROS) due to low expression of endogenous antioxidant proteins such as superoxide dismutases (SOD1 and SOD2) that convert superoxide into H₂O₂ and glutathione peroxidase (GPX1) and catalase (CAT) which dispose of H₂O₂ to water. ROS are commonly produced from nutrient metabolism in the mitochondria and are able to diffuse into the cytosol to act as secondary signals. Certain proteins, such as kinases, may be activated by ROS due to redox changes of cysteine residues and disulfide bonding. Here we determine glucose-produced H₂O₂ as a secondary signal of action on mTOR.

Materials and methods: Wistar rat islets were used for all experiments. Quantitative PCR measured mRNA, immunoblot measured relative protein expression, and enzyme activities were determined by specific assays. H₂O₂ was measured in islet cell lysates by Amplex Red fluorogenic activity. Adenoviral-mediated expression of CAT was used to rapidly dispose of H₂O₂. Phosphorylation activation of mammalian target of rapamycin (mTOR) and mTOR substrates - p70S6 kinase 1 & 2 (p70S6K), eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and protein kinase B (Akt) - was assessed with phospho-specific antibodies.

Results: The mRNA, protein, and activity of the major antioxidant enzymes in isolated rat islets relative to hepatocytes was measured. Islet mRNA levels for SOD1 were 89%, SOD2 55% and GPX1 96% lower than that in liver, with catalase mRNA undetectable in islets. However, protein levels for SOD1 were 40%, and SOD2 equivalent, to that in the liver. GPX1 activity was only 19% of liver, with no catalase activity present in islets. As such, β -cells can efficiently produce H₂O₂ from superoxide generated from oxidative metabolism but cannot efficiently dispose of H₂O₂. Glucose stimulation (16.7mM) of islets rapidly increased islet H₂O₂ 4-fold within 2 min. that was sustained over a 90-min. incubation. Adenoviral-mediated expression of CAT decreases H₂O₂ by >90% ($p=0.04$). The mTOR was used as a model nutrient-activated kinase to test if glucose-induced H₂O₂ is a metabolic signal to activate this enzyme. Two protein complexes contain mTOR - complex 1 (mTORC1) and 2 (mTORC2). Phosphorylation activation of mTORC1-specific substrates p70S6K and 4E-BP1 was examined in parallel to the specific mTORC2 substrate Akt. mTORC1 substrates are phosphorylated within the first 5 min. of glucose (16.7mM) stimulation and maintained over an hour, while insulin-like growth factor 1 (IGF-1) treatment only transiently phosphorylates mTORC1 substrates at 15 min. Activation of mTORC2 by IGF-1 occurs quickly and is sustained, but is only slight with glucose alone. These data indicate glucose specifically activates mTORC1. Furthermore, ablating H₂O₂ production by CAT expression significantly decreased glucose activation of mTORC1 by 36% ($p=0.03$) in islet β -cells, whereas mTORC2 remained unaffected.

Conclusion: The antioxidant system of the islet β -cell is coordinated to generate H₂O₂ in response to glucose oxidative metabolism. H₂O₂ is a candidate secondary signal for nutrient activation of mTORC1, but not mTORC2 in β -cells. The N-terminus of mTOR contains conserved cysteines that are ideal targets for oxidation by H₂O₂, and we are currently conducting site-directed mutagenesis on mTOR to pursue this idea.

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Expression and roles of NOX family NAD(P)H oxidases in pancreatic islets of Nox-specific knockout mice

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Background and aims: Elevated production of reactive oxygen species (ROS) has been proposed to contribute to pancreatic beta-cell impairment in diabetes. Expression of phagocyte-like Nox family NAD(P)H oxidases, a potential source of ROS, has been reported in pancreatic beta-cells. However, their role remains poorly characterized due to lack of specific inhibitors and

reliable antibodies. Here, functional analysis of Noxs in islets was carried out using Nox isoform-specific knockout mice.

Materials and methods: Expression of NAD(P)H oxidase components in C57BL/6J mouse and human islets was analyzed by RT-PCR. Next, islets isolated from wild-type (WT) mice and mice knocked out for specific Nox isoforms (koNox-1, -2 and -4) were used to study putative correlation between glucose-stimulated insulin secretion (measured by RIA) and Nox-derived superoxide generation (measured by NBT). Additionally, Nox-2 was knocked down in WT islets (siNox-2). Sub-cellular localization of NOX-2 in human islets was investigated (immuno-staining and internalization of rhodamine-conjugate dextran). KoNox-2 islet morphology, beta-cell area and insulin contents were measured (immuno-staining), calcium response was tested using fura-2-AM, and glucose homeostasis was examined by ipGTT. Additionally, specificity of the NOX inhibitor DPI was challenged on WT and koNox-1, -2 and -4 islets. The study has been carried out along the “Principles of laboratory animal care”.

Results: Both mouse and human pancreatic islets expressed membrane-associated NOX-2 and -4, NOX-2 being the predominant isoform. Nox-1 and cytosolic components, such as p22^{phox}, p40^{phox}, p47^{phox}, p67^{phox}, Noxo-1, Noxa-1 were expressed in mouse islets. Expression of Nox-2 was further confirmed in sorted beta-cells of mouse islets. A negative correlation ($r=-0.96$, $p<0.05$) was found between glucose-induced insulin secretion and Nox-derived superoxide generation comparing WT and koNox-1, -2 and -4 islets. Compared to WT, koNox-2 islets stimulated with 22.8 mM glucose exhibited potentiated insulin release (+119%, $p<0.05$) associated with lower superoxide production (-37%, $p<0.05$), confirmed with *in vitro* knockdown of Nox-2 (si-Nox-2). In human islets, NOX-2 co-localized with both insulin granules and endosomes/lysosomes. No differences in cytosolic calcium, islet organisation, beta-cell area and insulin contents and glucose homeostasis were observed. The NOX inhibitor DPI exhibited a general inhibitory effect on insulin secretion in WT and all koNox islets, showing lack of specificity of this inhibitor.

Conclusion: NOX-2 is the most abundant NAD(P)H oxidase in human and mouse islets. NOX-2 is found in insulin granules and endosomes/lysosomes of human beta-cells. Nox knockouts represent a valuable experimental model for the study of NAD(P)H oxidases, the use of non-specific NOX inhibitors being misleading. NAD(P)H oxidases positively contribute to glucose-induced ROS generation and negatively regulate insulin secretion.

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FoxO feedback control of basal IRS-2 expression in pancreatic beta cells is distinct from that in hepatocytes

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Background and aims: Appropriate regulation of IRS-2 expression in pancreatic beta-cells is essential to adequately compensate for insulin resistance. In liver, basal IRS-2 expression is controlled via a temporal negative feedback of SREBP1 to antagonize FoxO1/FoxO3a at an insulin response element (IRE) on the IRS-2 promoter. It was examined if a similar mechanism controlled IRS-2 expression in beta-cells.

Materials and methods: IRS-2 mRNA and protein expression as well as IRS-2 gene promoter activity, were examined in isolated rat islets ± various inhibitors and adenoviral gene manipulation. Specific transcription factor association with the IRE on the IRS-2 promoter was examined by chromatin immunoprecipitation (ChIP) assay and their nuclear translocation by immunofluorescence. A direct *in vivo* effect of insulin on control of IRS-2 expression in liver and pancreatic islets was also examined.

Results: In IRS-2 promoter-reporter assays conducted in isolated islets, removal of the IRE decreased basal IRS-2 promoter activity in beta-cells by < 80%. Activation of IRS-signaling in isolated rat islets by insulin/IGF-1 (used as an experimental *in vitro* tool) or downstream constitutive activation of protein kinase-B (PKB) significantly decreased IRS-2 expression. In contrast, inhibition of phosphoinositide 3-kinase (PI3K) or PKB significantly increased IRS-2 levels in beta-cells. ChIP assays indicated that transcription factors FoxO1 and FoxO3a associated to the IRE on the IRS-2 promoter in beta-cells in a PI3K/PKB dependent manner, whereas others like SREBP1, TFE3 and ARNT did not. However, only FoxO3a, not FoxO1, was capable of

driving IRS-2 promoter activity via the IRE in beta-cells. In *in vivo* studies insulin was able to suppress IRS-2 expression via activation of SREBP1 in the liver, but this mechanism did not occur in pancreatic islets from the same animal.

Conclusion: The molecular mechanism for feedback control of IRS-signaling to decrease IRS-2 expression in liver and beta-cells are quite distinct with a predominant role played by FoxO3a in beta-cells.

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PRL-induced improvement of beta cell function: Is Cx36 a player?

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Background and aims: Pregnancy is the only physiological state in which pancreatic β-cell mass and function are naturally enhanced. The increase in plasmatic prolactin (PRL) that occurs during this period is thought to mediate these beneficial effects. It was already reported that insulin secretion is influenced by connexin 36 (Cx36), a membrane protein which forms gap junctions for the β-cell-to-cell exchange of cytosolic molecules. Therefore, the objective of this work is to investigate whether the beneficial effects of PRL on β-cells are mediated by Cx36, and whether this phenomenon can be used as a novel therapeutic approach to treat diabetes.

Materials and methods: PRL effects were evaluated on the mouse β-cell line (MIN6). MIN6 cells were exposed to PRL (500 ng/mL) for 24–48h, and the following parameters were evaluated: Cx36 expression (at the transcriptional and translational levels, as evaluated by RT-PCR and Western Blotting (WB), respectively), insulin release (by RIA) and cell-to-cell coupling (by fluorescent dye microinjection). The *in vitro* situation was correlated to an *in vivo* approach where 18th days pregnant C57BL6 mice and OFA rats (with physiologically increased PRL levels) were compared to non-pregnant animals, with regard to Cx36 localization and expression, both at mRNA and protein levels.

Results: PRL exposure slightly increased Cx36 mRNA expression. However, the protein levels, evaluated by WB, did not change. Strikingly, the number of Cx36-formed gap junctional plaques showed a 3-fold increase ($p < 0.05$) and the cell-to-cell coupling index (that correlates the coupling incidence of Lucifer Yellow with the area of dye spread) was also increased 2.7 times, indicating an increase in Cx36 function after PRL exposure. Consistent with this effect, PRL exposure also increased *in vitro* the number of insulin immunopositive cells and both basal and glucose-dependent insulin release. The increase in Cx36 plaques with PRL was also observed *in vivo*. In pregnant mice and rats, Cx36 increased 2- ($p < 0.05$) and 1.2-fold ($p < 0.01$), respectively, when compared to non-pregnant animals, as evaluated by immunostaining of β-cells in islet sections. Dye coupling of β-cells was also increased 2-fold in islets isolated from pregnant rats ($p < 0.05$).

Conclusion: The results suggest that Cx36 is one of the players mediating the beneficial effects of PRL on β-cell function, due to its influence on insulin secretion. The influence of this protein is being investigated in MIN6 cells and mice null for Cx36.

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Cocaine- and amphetamine-regulated transcript (CART) is expressed in human islets and adipose tissue and stimulates insulin secretion and modulates insulin action

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Background and aims: CART is an anorexigenic peptide expressed in brain areas controlling appetite. CART is also expressed in islet cells and CART KO mice exhibit impaired glucose tolerance due to islet dysfunction. Furthermore, CART is upregulated in islets of Type 2 diabetic (T2D) patients and rodent models of T2D and CART regulates islet hormone secretion in rodents. In this study we examined the effect of CART on insulin secretion in human islets and the impact of viral overexpression of CART on insulin secretion in clonal beta cells. Furthermore, CART-expression was examined in human and rodent white adipose tissue (WAT) and the effect of CART on lipolysis and glucose uptake was assessed in primary rat adipocytes.

Materials and methods: Human islets were cultured in 2.8, 8.3 and 16.7 mM glucose for 1 h with or without the addition of 10nM CART 55-102. Human CART was overexpressed in INS-1 (832/13) cells using adenoviral vectors. Insulin secretion was measured using ELISA. CART-expression in human and rodent white adipose tissue (WAT) was assessed by qPCR, and immunocytochemistry. Glycerol release and glucose uptake was examined in primary rat adipocytes.

Results: CART caused a 1.5-fold increase in glucose-stimulated insulin secretion (GSIS) in human islets, but had no effect at basal glucose levels. Viral overexpression of CART in INS-1 832/13 cells provoked enhanced GSIS and GSIS potentiated by cAMP secretagogues (IBMX, forskolin, and GIP). On the other hand, overexpression of CART had no effect when 35mM KCl was present. The effect on GSIS was blocked by H89 (Protein kinase A inhibitor), but not by U73122 (Phospholipase C inhibitor). The effect of CART-overexpression under elevated cAMP-levels was blocked by both H89 and U73122. CART mRNA and protein expression was evident in human, rat and mouse WAT. In primary rat adipocytes, CART potentiated isoprenaline-induced lipolysis and activation of hormone sensitive lipase (HSL). Furthermore, CART potentiated the inhibitory effect of insulin on lipolysis. Without isoprenaline, CART reduced insulin-induced activation of PKB and subsequent glucose uptake.

Conclusion: We conclude that 1) CART stimulates insulin secretion in humans. 2) Overexpression of CART enhance GSIS via protein kinase A and phospholipase C-dependent pathways in INS-1 832/13 cells. 3) CART is a novel constituent of human and rodent WAT. 4) CART modulates the action of insulin and isoprenaline on lipolysis and glucose uptake. Together these data suggests that CART is an important regulator of glucose metabolism in humans.

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domain as evidenced by PM-IRRAS and ATR. Using the hGH reporter gene assay in PC12 cells, we found that transiently expressed wild-type VAMP2 but not mutant VAMP2 reconstituted KCl-evoked secretion after knock-down of endogenous VAMP2. Analysis of single fusion events by evanescent wave microscopy in clonal INS1-E beta-cells using VAMP2-phluorin as reporter revealed again a large decrease in stimulated secretion when comparing mutant VAMP2 versus the wild-type.

Conclusion: Collectively our results demonstrate an unprecedented dynamics of the transmembrane domain of VAMP2 never observed previously for any other transmembrane domain. Our mutational analysis suggests that these characteristics may play an important role during insulin exocytosis most likely by disturbing the membrane bilayers and thus promoting their fusion.

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Structural dynamics and functions of the transmembrane domain of VAMP2 during exocytosis

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Background and aims: The regulated secretion of insulin by exocytosis from pancreatic beta-cells exerts a major role in glucose homeostasis. A set of highly conserved transmembrane or membrane-attached proteins, the so-called SNARE proteins, forms a complex that plays a central role during exocytosis and has been described as the minimal machinery for membrane fusion. In insulin exocytosis, the SNARE complex is composed of the plasma membrane proteins syntaxin 1 and SNAP25 as well as VAMP2 localized on the vesicle membrane. Whereas structure and function of the cytosolic domains of the SNARE proteins have been studied extensively, the structure and the potential role of their transmembrane domains in exocytosis are still poorly understood. We have therefore performed a structure-function analysis of the transmembrane domain of VAMP2.

Materials and methods: Structural analysis by Polarization Modulation Infrared Reflection Absorption Spectroscopy (PM-IRRAS) and Attenuated Total Reflectance (ATR) infrared spectroscopy was performed on peptides synthesized in-house or full-length recombinant proteins incorporated in lipid model bilayers. Phylogenetic analysis was conducted with Vector NTI. To study the effect of wild-type or mutant VAMP2 in cells, we have established a system based on silencing endogenous expression of VAMP2 (using a specific shRNA), re-expression of wild type or mutant shRNA resistant VAMP2 and a reporter gene assay. Secretion assays were performed in cells co-transfected either with cDNA encoding human-growth hormone or a pH-sensitive GFP attached to VAMP2 as reporter. Mutagenesis was performed by standard protocols. INS-1E or PC12 cells were cultured under standard conditions. Sub-cellular localisation was assessed by confocal immunomicroscopy.

Results: We observed that the transmembrane domain of the SNARE protein VAMP2 is able to switch reversibly from alpha-helices to beta-sheets in lipid bilayers depending on the peptide/lipid ratio (relative protein concentration). These structural changes may disturb the lipid organization during fusion where the local concentration of SNAREs is expected to be augmented. Phylogenetic analysis of VAMP transmembrane domains revealed the conservation of two small amino acids 3 residues apart, thus facing the same side of a helix and suggesting space constraints in this region. Their mutation to larger residues retarded structural conversion of the VAMP2 transmembrane

OP 19 Insulin therapy

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Splitting high dose of insulin and injecting it in two sites improves blood glucose control

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Background and aims: Increasing number of severely insulin resistant type 2 diabetes patients require extremely high doses of insulin (>2 IU/kg body weight) to control their blood glucose. As the greater the volume of injected insulin preparation, the poorer the absorption of the drug; therefore simple increasing the dose may fail to achieve the expected blood glucose control improvement. As U500 insulin preparation is not available in many countries, we conducted a prospective randomized study to assess the effect of a split-dose insulin on a long term metabolic control of type 2 diabetes.

Materials and methods: 31 type 2 diabetes patients (17 men, mean [±SD] age 54.3±6.1 years, BMI 34.3±5.2 kg/m², diabetes duration 11±4 years) were enrolled into the study. The main inclusion criteria were 1) being treated with multiple insulin dose regimen; 2) the need to inject >60 IU of insulin as a single dose at least once daily, and 3) concomitant poor metabolic control (HbA_{1c} >8%). The subjects with severe insulin resistance secondary to known cause i.e. infections, steroid use etc. were excluded from the study. 16 subjects were randomized into two-sites (TS) group i.e. they split each high (>60 IU) dose of insulin in two equal parts and gave them in two different symmetrical locations (i.e. on both sides of umbilicus or in both thighs etc.), while the other 15 patients (one-site group; OS) maintained their usual (all dose injected in one site) insulin dosage regimen. All other treatment procedures including repeated education and concomitant medications were similar in both groups. Hemoglobin A_{1c}, plasma lipids, hypoglycemia occurrence as well as patients' satisfaction were assessed with VAS scale (range 0-100) every three months for a 12-month period.

Results: Baseline HbA_{1c} was in TS and OS groups 10.3±1.8 and 10.0±1.6%, and mean daily insulin doses were 254±52 and 240±30 IU, respectively. HbA_{1c} value decreased after 3-month treatment period to 9.1±1.3% (p<0.05 vs baseline) in TS group and remained stable in OS group (9.7±1.5%). After 6 months HbA_{1c} values in TS and OS groups were 8.6±1.4 and 9.9±1.8%, and after 12 months - 8.8±1.4 (p<0.05 vs baseline) and 10.4±1.7%, respectively. Fasting plasma triglycerides at baseline improved in TS group (236±49 at baseline and 192±28 mg/dl after 12 months; p<0.01) while they remained stable in OS group (252±43 at baseline and 241±62 mg/dl after 12 months). No major events of hypoglycemia occurred in any subject during the study period. Mean treatment satisfaction score increased from 53±18 to 72±23 (p<0.05) in TS group, and it was unchanged in OS group (55±20 at baseline and 58±27 after 12 months).

Conclusion: In severely insulin resistant and poorly controlled type 2 diabetes subjects, in case of U500 insulin preparation unavailability, splitting high dose of insulin and injecting it in two sites may lead to a significant improvement of metabolic control of the disease and the increase in the patients' treatment satisfaction, without enhancing the risk of hypoglycemia.

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Metabolic control and hypoglycaemia during the first year of insulin treatment in patients with type 2 diabetes mellitus: a systematic review and meta-analysis of different insulin regimens

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Intensive insulin treatment in type 2 diabetes mellitus (T2DM) is accompanied by hypoglycemia. Aim was to define metabolic control and hypoglycemia during the first year of treatment as a function of treatment modalities. We considered 72 RCTs (22466 patients, lasting 12-52 weeks, published as full papers during years 1991-2009); we analyzed treatment duration, insulin regimen (basal, biphasic, prandial), glycemic target (aim), insulin doses, HbA_{1c} and its changes, hypoglycemia frequency. Estimates of insulin regimens on final HbA_{1c} (standardized mean difference, SMD) and hypoglycemia (Odds Ratio, OR), were calculated using Der Simonian and Laird models. From 1991 to 2009, aim (r=-.26, p=0.0015), basal (r=-.382, p=0.0001), and final (r=-.498, p=0.001) HbA_{1c} decreased. Final HbA_{1c} correlated with aim (r=.343, p=0.0001), regimen (r=.177, p=0.0293), dose (r=-.183, p=0.0279), that at step-

wise regression analysis, were predictors of final HbA_{1c} (r = .591, F = 24.14). Hypoglycemia frequency correlated with duration (r=.54, p=0.0001), aim (r=-.216, p=0.0213), dose (r=-.337, p=0.0003), that at stepwise regression, were predictors of hypoglycemia (r=.626, F=21.69). At meta-analysis (Table), basal vs biphasic, basal vs prandial, glargine and detemir vs comparators had less effect on HbA_{1c} and on hypoglycemia; within basal and within prandial regimens, new vs old analogs had more effect on HbA_{1c} and less on hypoglycemia; prandial was more effective than biphasic on HbA_{1c} with similar effects on hypoglycemia. In conclusion, hypoglycemia is a predictable effect of intensive insulin treatment in T2DM; regimens and analogs should be tailored to patients.

Table: meta-analysis of different insulin regimens and insulin analogs

Regimen (number of studies)	HbA _{1c} (SMD, 95% C.I.)	Hypoglycemia (OR, 95% C.I.)
Basal vs biphasic (14)	-0.24 (-0.10/-0.39) *	0.63 (0.49/0.81) *
Basal vs prandial (8)	-0.57 (-0.35/-0.78) *	0.37 (0.24/0.58) *
Biphasic vs prandial (9)	-0.65 (-0.25/-1.05) *	1.00 (0.54/1.86)
Within basal (new vs old analogs) (11)	0.06 (-0.07/0.19)	0.71 (0.59-0.85) *
Within prandial (new vs old analogs) (10)	0.21 (0.03/0.40) *	0.81 (0.63/1.03)
Glargine vs comparators (22)	-0.14 (-0.02/-0.15) *	0.74 (0.63/0.86) *
Detemir vs comparators (10)	-0.15 (-0.04/-0.25) *	0.56 (0.40/0.78) *

* statistically significant

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Buccal spray insulin: a new tool to treat subjects with impaired glucose tolerance

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Background and aims: In patients with impaired glucose tolerance (IGT), upon implementation of life style changes and metformin, a third returns to normal glucose tolerance, a third continues with IGT and the rest goes on to develop clinical type 2 diabetes. An increased risk for cardiovascular disease occurs in the latter two groups even though there is no progression to diabetes. A previous proof of concept study demonstrated that treatment with 12 puffs of buccal spray insulin was followed by a significant 29.6% decrease in mean plasma glucose at two-hours and a 26.8% decrease at three-hours. Primary endpoint of this study is the reduction of HbA_{1c} of 0.3 % at 6 month treatment between experimental vs control group. Secondary endpoints include the evaluation of production of antibodies against insulins (IA), changes in body weight, number of hypoglycaemic events.

Materials and methods: We have designed a randomized controlled trial in patients with IGT comparing buccal spray insulin (12 puffs per meal) plus physical exercise and diet (treatment group A, n=16, HbA_{1c} at entry 6.2% ± 0.4) vs. physical exercise and diet only (control group B, n=16, HbA_{1c} at entry 6.0% ± 0.3). HbA_{1c} levels, metabolic parameters and insulin antibodies were measured at baseline and every 3 months up to 6 months. Primary endpoint is the reduction of HbA_{1c} of 0.3% at 6 month treatment between the experimental and the control group. Secondary endpoints include the evaluation of antibodies against insulin (IA), changes in body weight and number of hypoglycaemic events.

Results: Subjects treated with buccal spray insulin achieved a significant reduction of HbA_{1c} compared to the control group (Δ HbA_{1c} 0' - 6 months -0.3% vs +0.09% p= 0.002). There was no significant difference in body weight and no hypoglycaemic or other adverse events were observed during the study period in both groups. No generation of IA was observed in subjects with IGT treated with buccal spray insulin.

Conclusion: These preliminary results indicate that buccal spray insulin is an effective treatment compared to diet + physical exercise in patients with IGT in reducing HbA_{1c} without adverse effects. A larger trial is required to demonstrate the long term effects of this therapy.

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Insulin degludec does not compromise efficacy or safety when given in a flexible once-daily dosing regimen compared to insulin glargine once daily at the same time each day in type 2 diabetesS.L. Atkin¹, S. C. Bain², S. Gough³, M.V. Shestakova⁴, I. Raz^{5,6}, L. Blonde⁷, L. Meneghini⁸, K. Begtrup⁹, T. Johansen⁹, K.I. Birkeland^{10,11}¹Michael White Diabetes Centre, Hull York Medical School, Hull, ²Abertawe Bro Morgannwg University Health Board, Singleton Hospital, Swansea,³Oxford Centre for Diabetes, Endocrinology and Metabolism, UK,⁴Endocrinology Research Center, Moscow, Russian Federation, ⁵Hebrew University of Jerusalem, ⁶Hadassah University Hospital, Jerusalem, Israel,⁷Ochsner Diabetes Research Unit, Ochsner Medical Center, New Orleans,⁸Miller School of Medicine, University of Miami, USA, ⁹Novo Nordisk A/S, Søborg, Denmark, ¹⁰Faculty of Medicine, University of Oslo, ¹¹Oslo University Hospital, Norway.

Background and aims: Insulin degludec (IDeg) is a new-generation, ultra-long-acting basal insulin that forms soluble multi-hexamers upon s.c. injection, resulting in a flat and stable glucose-lowering effect. This ultra-long and stable action profile may offer the opportunity to vary the time of administration from day to day thus allowing patients to adapt injection timing to suit their daily activities. The primary objective of this registration trial was to evaluate non-inferiority of IDeg dosed once-daily in a flexible regimen (IDeg Flex) as compared to insulin glargine (IGlar) dosed every day at the same time each day.

Materials and methods: In this 26-week, open-label, treat-to-target trial, people with type 2 diabetes were randomised to IDeg Flex (n=229) and instructed to alternate the timing of insulin administration to morning and evening, in effect creating 8–40 h intervals between doses, or IGlar (n=230) given daily, at the same time each day according to label. Insulin was added to existing OAD therapy (if any) and titrated to FPG <5 mmol/l (90 mg/dl). Mean baseline characteristics such as age (56.2 vs. 56.7 yrs), HbA_{1c} (8.5 vs. 8.4%), FPG (9.0 vs. 9.0 mmol/l), diabetes duration (10.8 vs. 10.8 yrs), and BMI (29.3 vs. 30.0 kg/m²) were comparable between IDeg Flex and IGlar groups, respectively.

Results: For both groups, 88% of participants completed the trial. At 26 weeks, IDeg Flex and IGlar reduced HbA_{1c} by 1.28 and 1.26 %-points, respectively (estimated treatment difference (ETD) IDeg Flex-IGlar: 0.04 %-points [95% CI: -0.12; 0.20]; non-inferiority was confirmed as the upper 95% CI limit was <0.4). Mean FPG at Week 26 was significantly lower for IDeg Flex (5.8 mmol/l) than IGlar (6.2 mmol/l) (ETD: -0.42 mmol/l [-0.82; -0.02] p=0.04). At 26 weeks, mean daily insulin doses were similar between groups. Rates of confirmed hypoglycaemia (PG <3.1 mmol/l (56 mg/dl) or severe) were similar for IDeg Flex and IGlar (3.6 vs. 3.5 episodes/patient-yr; estimated rate ratio (ERR) IDeg Flex/IGlar: 1.03 [0.75; 1.40], p=NS) as were rates of nocturnal confirmed hypoglycaemia (0.6 vs. 0.8 episodes/patient-yr; ERR: 0.77 [0.44; 1.35], p=NS). Rates of adverse events were similar between groups. Severe hypoglycaemia was rare (2 episodes/group).

Conclusion: By using extreme dosing intervals of between 8 and 40 hours, this trial shows that IDeg can be dosed flexibly at any time of day so that injection times can be changed from day to day without compromising glycaemic control or safety compared to the commonly used basal insulin, IGlar, dosed at the same time each day according to label. The larger reduction in FPG with IDeg Flex, observed at similar rates of nocturnal hypoglycaemia to IGlar, may be related to the stable action profile of IDeg.

Clinical Trial Registration Number: NCT01006291

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Factors associated with weight gain in type 2 diabetic patients starting on insulin: the CREDIT studyB. Balkau¹, P.D. Home², E. Wang³, M. Marre⁴¹INSERM CESP U1018, Villejuif, France, ²INSERM CESP U1018, Newcastle University, UK, ³sanofi-aventis, Villejuif, ⁴INSERM U695, Paris, France.

Background and aims: Moderate weight gain is usual after starting insulin therapy. The identification of factors associated with weight gain may help to target prevention strategies for weight gain.

Materials and methods: In the 314-center, 12 country, non-interventional CREDIT study, data was available on the change in weight, one year after insulin initiation in 2442 type 2 diabetic patients. Factors associated with a weight gain ≥ 1.6 kg (the median), both at baseline and at follow-up, were compared.

Results: Fifty-two percent of participants began insulin treatment with basal insulin alone, 23% pre-mix insulin, and 25% other insulins; these percentages were 41, 27 and 30% at one year. Patients who gained weight had a higher HbA_{1c} at baseline, a lower BMI, fewer took concomitant glucose-lowering medications, fewer started basal insulin, but more were on a multiple injection regimen (Table). Higher insulin dose at one year was also associated with weight gain, and those who gained weight still had a slightly higher HbA_{1c}. There were differences between the countries in weight gain (P < 0.001), ranging from 34% of people in Croatia gaining > 1.6 kg to 72% in Portugal. Age, sex, smoking status, lipid profile, presence of micro-or macro-vascular disease and prior treatment for diabetes were not associated with weight gain.

Conclusion: In real-life clinical practice, factors others than those traditionally associated with weight gain on starting insulin are significant, and could be used to target those at particular risk. Patient characteristics associated with a one year weight gain ≥ 1.6 kg after insulin initiation:

Measures	Weight gain < 1.6 kg	Weight gain ≥ 1.6 kg	P
At insulin initiation			
HbA _{1c} (%)	9.2 (1.8)	9.8 (2.0)	< 0.001
BMI (kg/m ²)	30.1 (6.3)	28.4 (5.9)	<0.001
Other hypoglycemic treatment for diabetes	72%	66%	<0.001
Basal insulin	54%	47%	
Premix insulin alone	23%	24%	0.003
Other insulin	23%	29%	
Intensified treatment	23%	28%	0.004
At one year			
HbA _{1c} (%)	7.6 (1.4)	7.8 (1.3)	0.004
Daily doses (IU)	31.2 (21.0)	38.9 (25.1)	<0.001

Supported by: sanofi-aventis

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Is there still a place for human pre-mixed insulin in modern type 2 diabetes therapeutics?P. Mader¹, L. Czupryniak², R. Papis³, P. Bijos⁴¹NZOZPD-I, Kłodzko, ²Department of Clinical Diabetology, Łódź, ³Diabetic Clinic, Włocławek, ⁴Bioton S.A., Ożarów Mazowiecki, Poland.

Background: Increasing number of type 2 diabetes patients are treated with analog insulins. In some countries human insulin has been withdrawn by the manufacturers from the market. Analog insulins while effective, are also more expensive than human insulin preparations, and therefore using only insulin analogs contributes to the rising costs of diabetes treatment. We conducted a multicentre nationwide prospective study assessing whether traditional human insulin premixed compound preparations still may play a role in the treatment of type 2 diabetes.

Material and methods: The study group comprised 3372 patients with type 2 diabetes (52% women, mean \pm SD) 63.0 \pm 9.4 years, body weight 83.8 \pm 13.3 kg, BMI 30.1 \pm 5.4 kg/m², diabetes duration 9.3 \pm 7.4 years) in whom treatment with premixed insulin preparations 30/70 (Gensulin M30) was initiated. The treatment efficacy and safety were assessed after 12 weeks. The subjects were treated to achieve fasting plasma glucose <120 mg/dl and 2-hour postprandial plasma glucose <160 mg/dl. Hypoglycemia was diagnosed in cases: Slight hypoglycaemia: an event self controlled by the patient. Mild-severe hypoglycaemia: an event requiring assistance of another person. Severe hypoglycaemia: an event requiring administration intravenous glucose, glucagons, or hospitalization with a measured plasma glucose concentration <55 mg/dl (3 mmol/l).

Results: Treatment with premixed 30/70 insulin significantly improved daily plasma glucose profile in the studied cohort (table), and HbA_{1c} decreased from 8.5 \pm 1.2 at baseline to 7.6 \pm 0.9% (p<0.001). Mean insulin dose at the follow-up visit was 23 \pm 7 IU before breakfast and 17 \pm 7 IU before dinner, in addition 19% of patients required short acting insulin dose (11 \pm 6 IU) before meal. Severe (i.e. requiring help from another person) hypoglycaemia was noted in 31 patients (0.9%), and mild episodes of hypoglycaemia occurred in 657 patients (19.5%). Percentage of the patients declaring good health status increased from 50 at baseline to 62% at the follow-up, very good - from

6 to 12%, and percentage of the patients declaring moderate health status decreased from 35 to 22%, and poor - from 7 to 3% (all changes $p<0.05$). **Conclusion:** Introducing premixed human insulin 30/70 preparation is efficacious and safe in large majority of type 2 diabetes patients as it leads to significant improvement in metabolic control as well as better perception of the patients' own health status.

plasma glucose mg/dl	baseline	follow-up	p
fasting	159±39	127±25	<0.001
post-breakfast	191±47	152±29	<0.001
post-lunch	193±49	156±30	<0.001
post-dinner	188±47	150±29	<0.001

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OP 20 Regulation of hepatic glucose production

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Glucose appearance of large evening meals with low and high glycaemic load in type 1 diabetes

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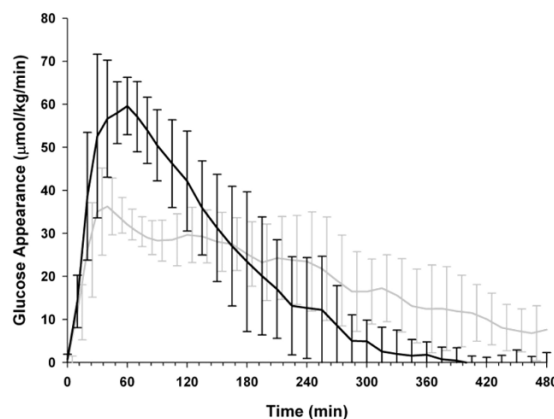
Background and aims: Insulin dose adjustment can be challenging for people with type 1 diabetes (T1D) when eating complex meals with large carbohydrate (CHO) content and different glycaemic load (GL). Using novel methodology, we estimated the systemic glucose appearance of two meals with identical CHO content but differing GL in young people with T1D.

Materials and methods: Sixteen subjects with T1D (age 19.5 ± 3.8 yrs, BMI 23.4 ± 1.5 kg/m², HbA_{1c} $8.7\pm1.7\%$, diabetes duration 9.0 ± 6.9 yrs, total daily insulin 0.9 ± 0.2 U/kg/day; mean±SD) were studied on two separate visits at a clinical research facility. On both visits, from 1000 until 1730, variable intravenous (iv) insulin was infused to achieve normoglycaemia. On Visit 1 all subjects fasted until 1800. Then eight subjects consumed a low GL pasta meal (CHO:protein:fat 120:35:30g; GL 54) and another eight subjects consumed a high GL potato meal (CHO:protein:fat 120:20:9g; GL 105), each enriched with [U-¹³C]glucose. Until 0200 next day iv insulin was infused to mimic prandial bolus of rapid-acting insulin (14 ± 2 U vs. 15 ± 4 U; low vs. high GL; $p=0.370$) and basal insulin delivery (0.9 ± 0.3 U/h vs. 0.9 ± 0.2 U/h; $p=0.850$). On Visit 2 identical iv insulin was given as on Visit 1 but, instead of the meal, variable iv 20% dextrose enriched with [U-¹³C] glucose was infused to reproduce the plasma glucose profile observed on Visit 1. Iv infusion of [6,6-²H₂] glucose and [U-¹³C;1,2,3,4,5,6,6-²H₇] glucose were given on both visits in a fashion to mimic endogenous glucose production (EGP) and glucose appearance of total CHO content from the meal (Ra_{meal}), respectively. Plasma glucose enrichment with the 3 tracers was measured by gas chromatography mass spectrometry. Total glucose appearance (Ra_{total}) on Visit 1 was estimated by double tracer approach. Ra_{meal} on Visit 1 was calculated as "Ra_{total} - EGP", where EGP was obtained from Visit 2 and estimated by the double tracer approach.

Results: Glucose appearance from low GL meal was less pronounced, presented a sustained plateau, and extended over the duration of the study, see Figure; 25% of the glucose from low GL meal appeared 88 ± 21 min after meal consumption, 50% at 175 ± 39 min, and 75% at 270 ± 54 min. Glucose from high GL meal appeared significantly faster with 25, 50 and 75% of the total appearance at 56 ± 12 ($p=0.009$), 100 ± 25 ($p=0.005$) and 153 ± 39 min ($p=0.003$) respectively. Bioavailability of total CHO did not differ between low and high GL meals (102 ± 14 vs $87\pm12\%$, $p=0.088$).

Conclusion: High GL large meal accelerates appearance of meal-derived glucose in the systemic circulation, whereas low GL meal results in reduced but sustained appearance. This suggests need for tailored prandial insulin delivery matching both CHO content and glycaemic load of the evening meal in T1D.

Figure. Rate of appearance of total carbohydrate content from a high glycaemic load meal (black line; 120g CHO, 105 GL) and a low glycaemic load meal (grey line; 120g CHO, 54 GL) [mean(SD)]



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Gluconeogenic glucose output could involve caveolin-1 dependent vesicles

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Background and aims: Endogenous glucose production is a crucial function maintaining glucose homeostasis. This function is composed of glycogenolysis and gluconeogenesis. According to the dogma, glucose is released via the glucose transporter Glut2. However, in Glut2 $-/-$ mice, a mechanism dependent on membrane traffic and able to compensate the absence of Glut2 has been evidenced. The aim of our study was to demonstrate the existence of a Glut2 alternative glucose output in wild type (WT) mice and characterize the vesicular pathway. Our hypothesis was that caveolin-1 dependent vesicles could play a role in glucose release in extracellular medium.

Materials and methods: Primary hepatocytes were isolated from fed and fasted C57/Bl6 mice (5 weeks males). Hepatocytes were incubated during 1h in DMEM 0g/L glucose and supplemented with lactate in the case of hepatocytes from fasted mice. Dynasore, a dynamin inhibitor was used at 80 μ mol/l and Cytochalasin B, a Glut2 inhibitor was used at 50 μ mol/l. Glucose release in the supernatant and intracellular glucose were measured by enzymatic assays. The same experiment was performed with hepatocytes from caveolin-1 $-/-$ mice (Cav1 $-/-$).

Results: We used hepatocytes from fed mice as a model of glycogenolysis and hepatocytes from fasted mice as a model of gluconeogenesis. To study the involvement of vesicles in glucose release we targeted dynamins which are known to be necessary to form the majority of vesicles. Glucose release from hepatocytes of fed mice was not inhibited by dynasore (48.2 \pm 5.6 vs 43.6 \pm 7.9 μ mol/l/million of cells/h). Glucose release from hepatocytes of fasted mice was decreased by 60% (22.9 \pm 3.1 vs 9.6 \pm 2.4 μ mol/l/million of cells/h, $p < 0.05$). In parallel, dynasore induced an accumulation of glucose within cells (2.3 \pm 0.2 vs 4.1 \pm 1.0 μ mol/l/million of cells, $p < 0.05$). These results suggest that a vesicular pathway might be implicated in gluconeogenic glucose output. Furthermore, glucose release from hepatocytes of fasted mice was nearly cancelled (3.1 \pm 0.7 μ mol/l/million of cells/h, $p < 0.05$) by a simultaneous treatment with dynasore and cytochalasin B, supporting the hypothesis of a complementary action of only two pathways: Glut2 and a vesicular output. Then, we used Cav1 $-/-$ mice to test the hypothesis that the vesicular pathway of glucose output could depend on caveolin-1. Glucose release from hepatocytes of fed Cav1 $-/-$ mice was equivalent to that of WT (37.4 \pm 8.1 μ mol/l/million of cells/h) and was not affected by dynasore treatment (40.4 \pm 7.1 μ mol/l/million of cells/h). Glucose release from hepatocytes of fasted Cav1 $-/-$ mice was 60% lower than that of WT mice (10 \pm 1.9 μ mol/l/million of cells/h, $p < 0.05$) suggesting that caveolin-1 was involved in glucose release. Moreover, dynasore had no effect on glucose release from hepatocytes of Cav1 $-/-$ fasted mice (7.6 \pm 1.6 μ mol/l/million of cells/h). In parallel to the lower glucose release in Cav1 $-/-$ hepatocytes, we measured an accumulation of intracellular glucose (4.0 \pm 0.6 μ mol/l/million of cells, $p < 0.05$), which was not further increased by dynasore treatment (4.9 \pm 0.8 μ mol/l/million of cells). These results suggest that gluconeogenic glucose output might be dependent on caveolin-1 and that this glucose output cannot be compensated by the glucose transporter Glut2. **Conclusion:** In conclusion, these results strongly suggest that a vesicular pathway is involved in the release of gluconeogenic glucose. Moreover, this Glut2 alternative pathway of glucose output would be dependent on caveolin-1, and would not be compensated by the presence of Glut2.

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Meal-induced insulin sensitisation depends on hepatic parasympathetic nerves and requires the presence of aminoacids and glucose in the gut

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Background and aims: Importance of analyzing postprandial glucose homeostasis has been highlighted by several studies suggesting that loss of postprandial glycemic control can precede deterioration of fasting glycemia in the course towards diabetes. Our group had previously reported that peripheral hypoglycaemic action of insulin increases from the fasted to the fed state and that this meal-induced insulin sensitization (MIS) mechanism is not triggered by glucose alone. We tested the hypotheses that the intestine plays an

essential role in activating MIS which is triggered by simultaneous presence of both carbohydrates and protein-based nutrients.

Materials and methods: 24 h-fasted Sprague-Dawley rats were used. Surgery involved gastric or enteric cannulation, and enteric band placement, to avoid intestine-stomach reflux. Three series of experiments were performed. 1) Insulin sensitivity (IS) was assessed by a rapid euglycaemic clamp both in fasting state (24 h-fast) and 120 min after administration of the liquid mixed-meal (carbohydrates, proteins and lipids, 10 ml/kg), either into the stomach (IG) or into the duodenum (IE). After hepatic parasympathetic inhibition (surgical ablation or atropine), IS was assessed. 2) Hepatic parasympathetic denervation was done in the fasted state; IS was assessed in fasted+denervated state and after the mixed-meal (IE). 3) The effect of meal composition on IS was studied by determining IS in the fasted state and after IE administration of the following liquid meals: (i) glucose + aminoacids + lipids (GAL); (ii) glucose + aminoacids (GA); (iii) aminoacids + lipids (AL); (iv) glucose + lipids (GL); (v) aminoacids (A).

Results: 1) IG mixed-meal did not produce MIS, unlike IE mixed-meal, which induced 77.4 \pm 11.2 % increase in IS (from 117.8 \pm 12.6 to 200.9 \pm 11.7 mg glucose/kg bw; $p < 0.001$ (fasting vs post-meal). Subsequent hepatic parasympathetic ablation reduced IS obtained after IE meal (denervation, 85.1 \pm 6.1 mg glucose/kg bw; atropine, 99.9 \pm 7.1 mg glucose/kg bw; $p < 0.001$ vs post-meal), but did not affect post-IG-meal IS. 2) When parasympathetic denervation was performed in the fasted state, it prevented IS increment after IE meal (3.8 \pm 13.3 %, ns). 3) The GAL meal induced significant insulin sensitization (MIS): IS increased from 97.9 \pm 6.2 mg/kg (fasted state) to 225.4 \pm 18.3 mg/kg ($p < 0.001$), which corresponds to 133.7 \pm 23.5 % potentiation of insulin action. The GA meal also produced MIS (IS increased from 115.3 \pm 15.3 to 241.6 \pm 35.2 mg/kg, after GA; $p < 0.05$), inducing 109.6 \pm 9.1 % potentiation of IS. None of the other meals tested produced a MIS close to the GAL or GA meals. Immediately before the post-meal IS assessment, insulinemias were similar in all groups. Furthermore, only GAL and GA meals induced sustained postprandial IS (higher than the fasting IS), as determined by a second post-meal IS test (data not shown).

Conclusion: Our data suggests that the MIS mechanism is triggered at the intestine and requires activation of the hepatic parasympathetic nerves. Glucose and aminoacids are required in the intestine in order to fully trigger the MIS mechanism.

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Altered glucose excursions during a meal tolerance test in an impaired hepatic vagal model are mainly due to peripheral insulin resistance

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Background and aims: In the last decades the hepatic parasympathetic system has emerged as a physiological modulator of insulin action having its main impact during the postprandial period. In the present work we investigated the hypothesis that the hepatic parasympathetic system is a determinant part in maintaining glucose and/or lipid homeostasis upon the ingestion of a meal. Moreover, the altered glucose excursions, as a consequence of the hepatic vagal impairment, are due to peripheral insulin resistance rather than to changes in insulin secretion/clearance or endogenous glucose production.

Materials and methods: Male Wistar rats (12 weeks old) were divided into two experimental groups: sham and hepatic parasympathetic denervated (HPNden) animals. A MTT was performed. The MTT consists in the intra enteric administration of a complete mixed meal: 38.2 mg/mL lipids; 84 mg/mL aminoacids; 173mg/mL glucose. To evaluate endogenous glucose production (EGP) a tracer - [U-13C] Glucose, representing 0.5% of the EGP - was administered as a continuous perfusion throughout the experiment. To assess glucose appearance rate, glucose clearance and glucose disposal the mixed-meal was enriched with a 5% [6,6-H2] Glucose as a tracer. Samples were evaluated by LC-MS (Liquid Chromatography - Mass Spectrometry). Plasma samples were collected at different times (0, 2, 5, 10, 20, 30, 40, 50, 60, 90 and 120 minutes) of the study. These samples were used to measure glycaemia, plasma insulin, c-peptide and free fatty acids. Insulin secretion was determined based on plasma c-peptide; insulin clearance was assessed as the AUC of [c-peptide]/[insulin]. Finally, insulin sensitivity was determined by a hyperinsulinemic euglycemic clamp.

Results: The results showed an increase in glucose excursions from the sham animals to the HPNden animals (AUC: 15650 ± 621.9 n=8 vs. 17980 ± 876.2 ; n=8; $P < 0.05$).

Insulin and c-peptide levels obtained for both groups were indistinguishable during the MTT, excluding insulin secretion as a contributor for the differences observed in the glycemic profiles. Likewise, insulin clearance and endogenous glucose production did not differ between both groups. Insulin sensitivity was evaluated before and after administration of the meal. The HPNden animals showed decreased postprandial insulin sensitivity, when compared with the control (113.4 ± 26.48 mg glucose/kg, n=4 vs. 203.4 ± 31.14 mg glucose/kg, n=4; $P < 0.05$).

Conclusion: The results indicate that in the absence of a fully functional hepatic parasympathetic system whole-body glucose homeostasis cannot be maintained. This leads to increased glucose excursions as a result of increased peripheral insulin resistance. Furthermore, while evaluating the pathways through which this system influences glucose homeostasis it was observed that pancreatic insulin secretion, insulin clearance, endogenous glucose production and plasma free fatty acids were not affected by hepatic vagal impairment throughout the MTT. These results allow us to conclude that the hepatic parasympathetic system is a critical component of postprandial glucose homeostasis.

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CITED2 links hormonal signals to PGC-1 α acetylation for regulating fasting gluconeogenesis

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Background and aims: In mammals deprived of food, induction of hepatic gluconeogenesis is important to ensure energy homeostasis in response to the energy demand. Such induction is dysregulated, however, in type 2 diabetes, resulting in the development of fasting hyperglycemia. Hormonal and nutrient regulation of metabolic adaptation during fasting is mediated predominantly by the transcriptional coactivator PGC-1 α in concert with various other transcriptional regulators including the transcription factors FoxO1, HNF-4 α , GR and PPAR α as well as the histone acetyltransferase CBP/p300. Although CITED2 (CBP/p300-interacting transactivator with glutamic acid- and aspartic acid-rich COOH-terminal domain 2) interacts with many of these molecules, the role of this protein in regulation of hepatic gluconeogenesis has been unknown. The aim of this study was to elucidate the role of CITED2 in hepatic gluconeogenesis.

Materials and methods: To investigate the role of CITED2 in gluconeogenesis, we conducted gain-of- and loss-of-function experiments using adenoviruses encoding CITED2 and CITED2 shRNA, respectively.

Results: Depletion of CITED2 in primary mouse hepatocytes by infection with an adenoviral vector encoding CITED2 shRNA attenuated the expression of the gluconeogenic genes G6pc and Pck1 as well as glucose production induced by cAMP. Conversely, forced expression of CITED2 augmented these effects of cAMP on gluconeogenic gene expression and glucose production. Knockdown of CITED2 in the liver of obese diabetic db/db mice significantly reduced both gluconeogenic gene expression in the liver during fasting as well as blood glucose concentrations under fed and fasting conditions. In contrast, overexpression of CITED2 in the liver of lean mice increased hepatic G6pc and Pck1 expression as well as blood glucose levels during fasting. We also examined the effect of CITED2 on gluconeogenic gene expression induced by PGC-1 α . Overexpression of CITED2 enhanced, but its deletion attenuated PGC-1 α -induced gluconeogenic gene expression, suggesting that CITED2 upregulates PGC-1 α activity. Next we investigated the mechanism by which CITED2 augments PGC-1 α activity. We found that CITED2 inhibited the acetylation of PGC-1 α by blocking its interaction with GCN5, a key acetyltransferase for PGC-1 α . The consequent reduction in the level of PGC-1 α acetylation resulted in an increase in its transcriptional coactivation activity and up-regulation of the expression of gluconeogenic genes. In addition, we found that the interaction of CITED2 with GCN5 was disrupted by insulin in a manner dependent on phosphoinositide 3-kinase (PI3K) signaling, whereas fasting glucagon leads to CITED2 induction.

Conclusion: CITED2 functions as a transducer of glucagon and insulin signaling in the regulation of PGC-1 α activity associated with the transcriptional control of gluconeogenesis, and that this function is mediated through modulation of GCN5-dependent acetylation of PGC-1 α .

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Inhibitors of endoplasmic reticulum stress prevent hepatic insulin resistance induced by prolonged lipid infusion

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Background and aims: To understand the mechanisms of fat-induced insulin resistance, we utilized the 48h Intralipid plus heparin (IH) infusion model, which leads to prolonged plasma elevation of free fatty acids. In this model, the anti-inflammatory sodium salicylate and the antioxidant N-acetyl-L-cysteine (NAC) prevented lipid-induced peripheral insulin resistance, but, in contrast to the 7h IH infusion model, did not prevent hepatic insulin resistance. Here we investigated whether the chemical chaperone 4-phenylbutyric acid (PBA), which alleviates endoplasmic reticulum stress, prevented hepatic and peripheral insulin resistance after prolonged (48h) lipid infusion.

Materials and methods: Cannulated Wistar rats (250-300g, n=5-9/group) were infused i.v. for 48h with Saline (5.5 μ l/min), IH (20% Intralipid + 20 U/ml heparin, 5.5 μ l/min), IH with PBA (2.08 μ mol kg⁻¹ min⁻¹), or PBA alone. After an overnight fast, a hyperinsulinemic (5 mU kg⁻¹ min⁻¹) euglycemic clamp was performed with tritiated glucose methodology to assess hepatic and peripheral insulin sensitivity.

Results: Insulin-mediated suppression of endogenous glucose production (EGP), expressed as percentage of basal EGP, was impaired by IH (10 \pm 9% in IH vs. 57 \pm 11% in Saline and 55 \pm 9% in PBA alone, $p < 0.05$) and at least partially restored by PBA co-infusion (40 \pm 6%). Insulin-stimulated glucose utilization was blunted by IH, but this impairment was completely prevented by PBA (Saline, 29.3 \pm 0.6 mg kg⁻¹ min⁻¹; IH, 24.3 \pm 1.0 mg kg⁻¹ min⁻¹; IH+PBA, 29.9 \pm 1.0 mg kg⁻¹ min⁻¹; PBA alone, 31.6 \pm 1.3 mg kg⁻¹ min⁻¹; $p < 0.05$ for IH vs. Saline, IH+PBA, and PBA alone).

Conclusion: In contrast to our previous studies involving sodium salicylate and NAC, the results herein indicate that PBA is effective at preventing both hepatic and peripheral insulin resistance in our model of prolonged lipid infusion. Since this chaperone is safe for human use, it may represent a new and upcoming therapy for fat-induced insulin resistance at the level of the liver and periphery.

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OP 21 Novel cardiovascular biomarkers

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Osteoprotegerin is increased in patients with type 1 diabetes and vascular complications and predicts cardiovascular mortality

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Background and aims: The bone-related peptide osteoprotegerin (OPG) is produced by vascular cells and is involved in the process of vascular calcification. We investigated the effect of serum OPG concentration on the prediction of cardiovascular disease (CVD) events, deterioration of diabetic nephropathy (DN) and all-cause mortality in patients with type 1 diabetes (T1D). We further examined whether OPG is associated with other vascular complications such as retinopathy and arterial stiffness.

Materials and methods: Serum OPG was measured by a Time-Resolved Immunofluorometric Assay (TRIFMA) in 2,116 patients with T1D and in 212 healthy control subjects participating in the Finnish Diabetic Nephropathy (FinnDiane) study. Pulse-wave analysis with augmentation Index (AIx) measurement was performed in 180 patients with T1D and 112 controls. Data on progression of renal disease, incidence of CVD and cardiovascular as well as all-cause mortality were verified from medical files and patients were followed for 5.4 ± 2.0 (mean ± SD) years.

Results: Patients in the highest (fourth) OPG quartile were at a significantly higher risk for all-cause mortality than patients with low levels (first quartile) (covariate-adjusted hazard ratio [HR] 1.01 [1.01-1.01]; $P < 0.001$). High OPG also predicted an incident CVD event independently of clinical CVD risk factors [HR 1.02 (1.00-1.02); $P = 0.007$]. In addition, OPG predicted progression to ESRD during follow-up [1.02 (1.00-1.03); $P = 0.03$ with adjustment with age, gender, SBP, total cholesterol, HbA_{1c}, smoking, and previous CVD]. Serum OPG was significantly increased in the patients with diabetic nephropathy (in patients with macroalbuminuria; $2,073 \pm 78$ ng/l or ESRD; $2,700 \pm 144$ ng/l) than in patients without DN ($1,607 \pm 40$ ng/l) or healthy controls ($1,626 \pm 55$ ng/l). Serum osteoprotegerin was also significantly higher in patients with T1D who had diagnosis of CVD (coronary heart disease, stroke or amputation) than those without ($2,449 \pm 140$ ng/l vs $1,683 \pm 35$ ng/l, $P < 0.001$). The patients with laser-treated retinopathy had significantly increased OPG levels. OPG was positively associated with arterial stiffness as measured by AIx both in patients with T1D ($r = 0.27$, $P < 0.001$) as well as in controls ($r = 0.23$, $P < 0.05$).

Conclusion: In a large contemporary cohort of individuals with T1D, OPG was not only predictive of CVD events and progression of kidney disease but also of all-cause mortality. OPG levels were strongly associated with diabetic complications such as retinopathy and arterial stiffening.

Supported by: Finnish Cultural F, Finnish Diabetes Research F, Folkhälsan Research F.

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Serum peroxiredoxin-4 and mortality in patients with type 2 diabetes (ZODIAC-28)

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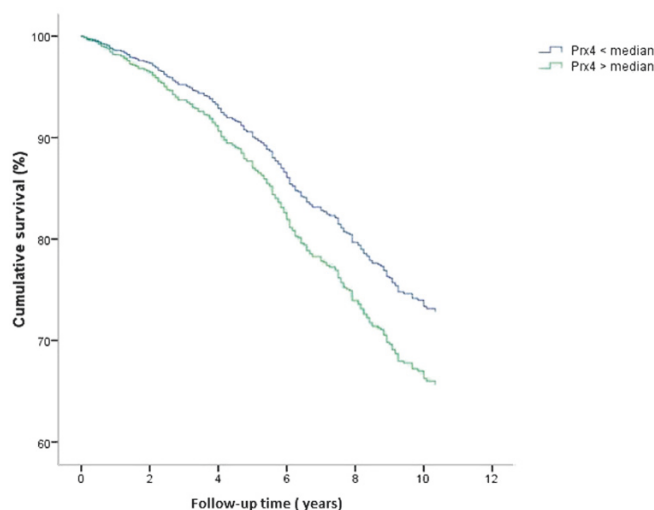
Background and aims: Hyperglycaemia induces oxidative stress and endothelial damage leading to micro- and macrovascular complications in patients with diabetes. Recently, circulating levels of Peroxiredoxin 4 (Prx4) have been proposed as a novel biomarker of oxidative stress. We prospectively

investigated whether circulating levels of Prx4 predict mortality in patients with type 2 diabetes.

Materials and methods: This study is part of the ZODIAC study, a prospective observational study in primary care patients with type 2 diabetes from the Netherlands and incorporates two cohorts: one started in 1998 and the other in 2001. Prx4 was measured with an immunoluminometric sandwich assay in 2010. A Cox proportional hazard model was used to investigate the relationship of Prx4 with cardiovascular and all-cause mortality, before and after adjustment for potential confounders. Three models were chosen: model 1 included Prx4 without confounders, model 2 included age and gender as confounders, and model 3 included BMI, serum creatinine, smoking, diabetes duration, systolic blood pressure, cholesterol-HDL ratio, history of macrovascular complications, and albuminuria as additional potential confounders. Prx4 and serum creatinine were logarithmically transformed because of skewed distribution.

Results: Prx4 was assessed in baseline serum samples of 1067 patients [age 67.2 ± 11.4 years, 485 (45.5%) males. Median [interquartile range] Prx4 was 0.86 [0.61 - 1.34] U/L. Further baseline data include: median HbA_{1c} 53 [45 - 66] mmol/mol, diabetes duration 4 [2 - 9] years, estimated creatinine clearance 72 [57 - 91] ml/min and urinary albumin creatinine ratio 1.97 [0.92 - 7.01] mg/mmol. After a median follow-up period of 9.8 years, 344 (32.3%) patients had died, of which 148 deaths (43.0%) were attributable to cardiovascular causes. Increased levels of Prx4 were associated with high rates of cardiovascular and all-cause mortality [hazards ratios (HRs) (95% confidence interval) were 1.83 (1.46 - 2.30) and 1.80 (1.53 - 2.12), respectively]. Adjusted HRs (95%CI) for cardiovascular and all-cause mortality were 1.64 (95%CI 1.27 - 2.13) and 1.59 (95%CI 1.31 - 1.91) in model 2 and 1.52 (95%CI 1.13 - 2.04) and 1.51 (95%CI 1.24 - 1.82) in model 3, respectively. The figure shows adjusted survival curves according to Prx4 levels.

Conclusion: In this prospective study we found that increased levels of Prx4 are independently associated with increased risk of cardiovascular and all-cause mortality in patients with type 2 diabetes.



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Circulating adrenomedullin levels are predictive of mortality and hospitalisation in heart failure patients with and without diabetes mellitus

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Background and aims: Adrenomedullin is a vasoactive peptide, which is a potential biomarker of vascular injury in patients with cardiovascular disease and with diabetes mellitus. The aim of the present study was to investigate whether mid-regional pro adrenomedullin (MR-proADM) is predictive of outcome in patients with and without diabetes mellitus from an outpatient heart failure clinic.

Materials and methods: Prospective observational study of 366 unselected patients included at the baseline visit (31 % female, mean age 70 years), 62 % had ischemic heart disease, 19 % had a confirmed diagnosis of diabetes mellitus and were treated with anti-diabetic therapy. Patients were followed for a median of 55 months with respect to mortality and a combined endpoint of mortality or hospitalisation. A total of 189 of the patients died and 232 were hospitalized.

Results: At baseline mean (SD) MR-proADM levels were elevated in patients with diabetes and nephropathy (urinary albumin/creatinin ratio ≥ 30 mg/g) as compared with diabetic patients and patients without diabetes (1.12(0.86) vs. 0.84(0.54) vs. 0.75(0.43) nmol/l, $P=0.014$). Plasma MR-proADM was associated with increasing age ($r=0.28$, $P<0.001$), s-creatinine ($r=0.36$, $P<0.001$), NYHA-class ($r=0.14$, $P=0.007$) and with NT-proBNP ($r=0.32$, $P<0.001$). However, no relation with metabolic parameters such as HbA1c, BMI, HDL-cholesterol or triglycerides was observed. Using Cox proportional hazard analysis increasing levels of logMR-proADM was predictive of death Hazard ratio (HR) being 1.5 (95 % CI: 1.2–1.8, $P<0.001$) and of death or hospitalization HR 1.3 (1.1–1.4, $P=0.06$) per 1 SD increase adjusted for age and sex. The prognostic ability of MR-proADM with respect to overall mortality was not significantly attenuated after adjustment for s-creatinine, presence of diabetes, ischemic heart disease, NYHA functional class, or NT-proBNP HR 1.3 (1.1–1.5, $P=0.004$) however, with respect to the combined endpoint of death or hospitalization HR 1.1 (0.9–1.4) remained no longer significant. In patients with diabetes MR-proADM levels was equally predictive of death HR 1.5 (1.1–2.1, $P<0.001$) adjusted for age and sex, as compared with the total cohort. **Conclusion:** MR-proADM levels are elevated among cardiac patients with diabetes and nephropathy. This biomarker of vasculopathy predicts mortality independently of presence of diabetes mellitus, ischemic heart disease and kidney function and could be a novel biomarker of outcome with incremental prognostic information to traditional clinical risk factors and NT-proBNP in cardiovascular disease and in patients with diabetes mellitus.

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Serum resistin is associated with coronary artery disease and predicts major cardiovascular events in type 2 diabetes patients

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Background and aims: High serum resistin has been associated with increased risk of cardiovascular events in the general population. At variance, only two small studies with conflicting results have been reported in patients with type 2 diabetes (T2D), of Asian ancestry. The aim of this study was to deeply investigate the role of serum resistin levels on cardiovascular disease in large samples of patients with T2D of European ancestry.

Material and methods: To accomplish this aim the following studies were carried out. (1) A case-control study for coronary artery disease (CAD) (from Gargano, Italy; Gargano Heart Study: GHS-cross sectional design) in 360 with angiographically diagnosed CAD - CAD positive - and 416 with no evidence of CAD - CAD negative - patients with T2D; (2) a case-control study for CAD (from Boston, MA; Joslin Heart Study - JHS) in 418 CAD positive and 433 CAD negative patients with T2D; (3) a prospective study for major cardiovascular events including cardiovascular death, non fatal myocardial infarction - MI - and non fatal stroke (from Gargano; Gargano Heart Study: GHS-prospective design) in 340 patients with T2D and CAD, so far followed up for 1-91 months. Serum resistin levels were measured by ELISA in all 1,977 participants.

Results: Compared to CAD negative, serum resistin concentrations were higher in CAD positive patients in both GHS-cross sectional (9.33 ± 5.03 vs. 10.72 ± 6.68 ng/ml, age, sex and smoking habit adjusted $p=0.02$) and JHS (6.46 ± 4.25 vs. 8.64 ± 5.99 ng/ml, adjusted $p<0.001$) patients. Among the participants to GHS-prospective, 32 cardiovascular deaths, 3 non fatal MIs and 8 non fatal strokes occurred during follow-up (annual incidence rate 0.04). Patients who had resistin levels above the median of the entire cohort (> 8.9 ng/ml) had a significantly increased risk of major cardiovascular events (adjusted HR=3.25; 95% CI: 1.58–6.68 $p=0.001$). These associations were unaffected by further adjustment for BMI, duration of diabetes and current therapy.

Conclusion: Elevated serum resistin concentration is a strong and independent risk factor for cardiovascular disease in patients with T2D. This points to resistin as a novel biomarker which might be useful in the clinical set in improving predictability of cardiovascular disease in patients with T2D.

Supported by: EFSD/Pfizer grant

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Complement activation and prognosis in patients with myocardial infarction and type 2 diabetes: a report from the DIGAMI 2 trial

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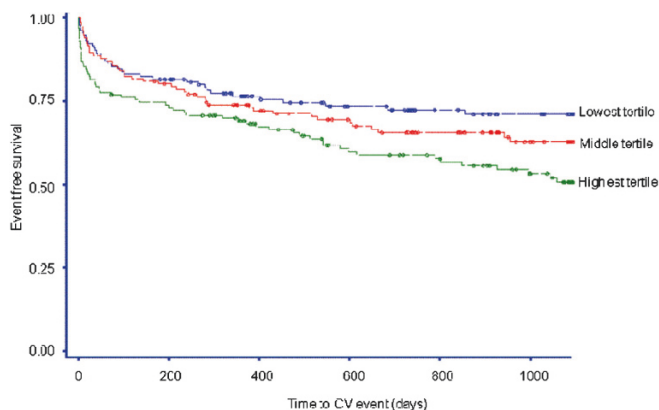
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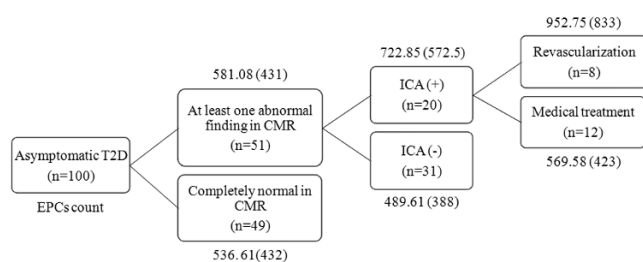
Background and aims: Activation of the complement system may enhance the negative impact of ischemia followed by reperfusion, but information on the effect of different levels of this system is conflicting. We characterized the complement end-product sC5b9 and the mannan-binding lectin associated serine protease 2 (MASP-2) in patients with type 2 diabetes (DM) and myocardial infarction (MI) with the aim to explore their potential as prognostic markers.

Materials and methods: Plasma-sC5b9 and MASP-2 was, at the time for hospital admission, determined in 391 patients with MI and DM (median age 70 years; male 67%). Adjudicated endpoints were cardiovascular events (CVE= cardiovascular mortality and nonfatal MI or stroke). **Results:** Median sC5b9 was 134 ng/mL (interquartile range (IQR) 101 - 190 ng/mL) and median MASP-2 333 μ g/L (IQR 235 - 463 μ g/L). There was no significant correlation between sC5b9 and MASP-2. Women had significantly higher sC5b9 than men (median 152 vs 130 ng/mL; $p=0.02$). Both sC5b9 and MASP-2 correlated to age and creatinine clearance. Furthermore MASP-2 correlated with BMI. During 2.3 years CVE occurred in 138 patients (35%). Both sC5b9 (HR 1.37, 95% CI 1.13 - 1.65; $p<0.01$) and MASP-2 (HR 0.68 (95% CI 0.51- 0.92; $p=0.01$) predicted CVE in unadjusted analyses. In the final models adjusting for age, creatinine clearance, BMI and previous MI and for MASP-2 also for admission glucose the predictive capacity remained for sC5b9 (HR 1.32, 95% CI 1.03-1.70; $p=0.03$) but not for MASP-2 (HR 0.86; 95%CI 0.62-1.20; $p=0.37$). The prognosis was less favourable in patients belonging to the highest sC5b9 tertile (logrank test $p=0.003$; Figure). **Conclusion:** In MI patients with DM high levels of sC5b9 predicted future CVE while the impact of MASP-2 was blunted by traditional risk markers. The prognostic implication of C5b9 deserves further studies exploring if mechanistic insights may help to improve the outcome for DM patients with MI.

Figure: Kaplan-Meier curves for cardiovascular (CV) events by C5b9 tertiles (logrank test $p=0.003$)



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Increased EPCs level can help the clinical decision on coronary intervention in asymptomatic type 2 diabetic subjects with abnormal finding in cardiovascular MRH. Kim¹, B.-W. Lee¹, J. Moon¹, M. Chae², H. Seok¹, Y. Kim³, H.-J. Chang¹, B. Cha¹, H. Lee¹;¹Department of Internal Medicine, Yonsei University College of Medicine, Seoul, ²Brain Korea 21 project for Medical Science, Yonsei University College of Medicine, Seoul, ³Department of Radiology, Yonsei University College of Medicine, Seoul, Korea, Republic of.**Background and aims:** We investigated endothelial progenitor cells (EPCs) and other risk factors for predicting occult coronary arterial disease (CAD) in asymptomatic subjects with type 2 diabetes (T2D) using cardiovascular MR (CMR).**Materials and methods:** We conducted a clinic-based, prospective study of asymptomatic patients with T2D. A total of 100 subjects (51 men and 49 women; mean age, 56.4 ± 7.6 years) were finally enrolled in this study. Clinical and laboratory parameters, including EPCs (CD34⁺/CD133⁺/CD309⁺) counts, and functional stress myocardial perfusion and anatomic image of coronary arteries using CMR were evaluated. Within 90 days after CMR, invasive coronary angiography (ICA), as determined a priori by the responsible cardiologist, was performed.**Results:** Total 51 patients had at least one abnormal finding in CMR (3 silent myocardial infarction, 11 inducible ischemia, 37 suspected CAD). ICA was performed in 20 patients. Eight of the 20 patients had significant CAD that was treated with revascularization (7 percutaneous coronary intervention and 1 coronary artery bypass graft). Compared to subjects without revascularization, patients treated with revascularization had significantly increased waist/hip ratio (0.90 ± 0.56 vs. 0.96 ± 0.07 , $p = 0.006$), HbA1c (7.0 ± 0.9 vs. 7.8 ± 1.4 , $p = 0.022$), and high EPCs level (525.08 (429.5) vs. 952.75 (833), $p = 0.033$). Interestingly, EPCs level was higher in patients who underwent ICA than in patients who were recommended observation (722.85 (572.5) vs. 489.61 (388), $p = 0.127$), and it was also increased in patients who had revascularization compared with the patients who were treated medically (952.75 (833) vs. 569.58 (423), $p = 0.157$) (Figure 1). Binary logistic regression analysis revealed that a high EPCs level and a high BMI were independently correlated with significant CAD requiring revascularization in patients who had abnormal findings in CMR. **Conclusion:** In this present study, obesity, control status of diabetes and EPCs count were associated with revascularization in asymptomatic patients with T2D. Considering the result of CMR, EPCs level was increased in patients who needed revascularization and it can help the clinical decision on coronary intervention in asymptomatic T2D subjects with abnormal finding in CMR.**Figure 1.** EPCs increased in patients who underwent ICA, especially with significant CAD. Data are presented as medians. EPCs = endothelial progenitor cells; ICA = invasive coronary angiography; CAD = coronary artery disease; T2D = type 2 diabetes; CMR = cardiovascular magnetic resonance.

Supported by: MEST

OP 22 Gastro-entero-pancreatic hormones

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The type 2 diabetes-associated zinc transporter ZnT8 is required in alpha cells for the normal stimulation of glucagon release in response to hypoglycaemiaG. Meur¹, S. Migrenne², E.A. Bellomo¹, P.L. Herrera³, C. Magnan², G.A. Rutter¹;¹Division of Medicine, Imperial College, London, UK, ²Université Paris Diderot, France, ³Department of Cell Physiology and Metabolism, University of Geneva, Switzerland.**Background and aims:** ZnT8 (SLC30A8) is a largely endocrine pancreas-restricted member of the zinc transporter (ZnT/slc30) family present in both β and, at lower levels, in α cells. Polymorphisms in the human SLC30A8 gene are associated with an increased risk of type 2 diabetes in man and mice inactivated for slc30a8 either systemically or in the β cell display abnormal glucose homeostasis. Whilst mice inactivated for ZnT8 selectively in the α cell display normal glucose tolerance, glucagon secretion in these animals in response to hypoglycaemia has not been previously examined.**Materials and methods:** Mice carrying floxed slc30a8 alleles on a mixed C57BL/6/sv129 background were crossed with animals expressing Cre recombinase under a 0.6 kb fragment of the preproglucagon promoter. Mice (10–14 weeks of age) homozygous for floxed alleles of slc30a8 and bearing the Cre transgene were compared to wild type or heterozygous control mice during hypoglycaemic clamp. Briefly insulin was infused through fixed catheters simultaneously with glucose to achieve levels of glycaemia of 2–2.5 mM for 90 min. Glucagon secretion from islets isolated by collagenase infusion was assessed during static incubations (30 min.) and quantified by radioimmunoassay.**Results:** Whereas fasting glucose concentrations were similar between genotypes, glucose-infusion rates required to maintain hypoglycaemia in α ZnT8 KO mice were significantly ($24 \pm 5\%$; $p < 0.01$ and $20 \pm 3\%$, $p < 0.05$ at 20 and 40 min. after the onset of glucose/insulin infusion; $n = 5$ –6 mice per condition) lower compared to wild-type mice. Measured at the end of the clamps (90 min.), plasma glucagon levels were elevated in α ZnT8 KO (620 ± 50 pg/ml) vs control (320 ± 60 pg/ml, $p < 0.05$) mice. By contrast, examined in isolated islets, decreased glucose concentrations (0.5 or 3 vs 17 mM) stimulated glucagon secretion significantly ($p < 0.05$) more weakly from α ZnT8 KO (0.05 ± 0.01 and $0.01 \pm 0.02\%$ of glucagon content / 30 min. respectively) than wild-type islets (0.15 ± 0.05 and $0.05 \pm 0.008\%$). No significant difference in glucagon release was apparent between genotypes at 17 mM glucose (0.02 ± 0.015 and $0.01 \pm 0.01\%$ released). Total glucagon content was higher in α ZnT8 KO islets (3.3 ± 0.05 ng/islet) than controls (2.05 ± 0.2 ng/islet).**Conclusion:** These findings demonstrate a previously-unexpected role for ZnT8 in the islet α cell. Importantly, we show that inhibition of ZnT8 selectively in these cells enhances glucagon release *in vivo* to enhance counter-regulatory responses. Since these changes were not, however, reflected by enhanced secretion *in vitro* in response to glucose deprivation, it seems likely that other mechanisms, possibly including enhanced responses to other stimuli (eg adrenaline) may mediate the enhanced release *in vivo*. Pharmacological modulation of ZnT8 activity may thus provide a novel means to control glucagon release in the context of type 1 and/or type 2 diabetes.

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Impaired incretin-induced amplification of insulin secretion following glucose homeostatic dysregulation in healthy subjectsK.B. Hansen^{1,2}, T. Vilbøll³, J.I. Bagger³, J.J. Holst², F.K. Knop³;¹Clinical Physiological, Glostrup Hospital, ²Biomedical Sciences, Panum Institute; University of Copenhagen, ³Internal Medicine F, Gentofte Hospital, Hellerup, Denmark.**Background and aims:** Type 2 diabetes is characterised by impaired insulinotropic effect of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). It remains unclear whether this impairment is a primary pathophysiological defect or whether it occurs as a consequence of glucose intolerance. Therefore, we aimed to inves-

tigate the insulinotropic effect of GIP and GLP-1 compared to placebo before and after 12 days of glucose homeostatic dysregulation in healthy subjects.

Materials and methods: The insulinotropic effect was measured using hyperglycaemic clamps and infusion of physiological doses of GIP, GLP-1 or saline in 10 healthy Caucasian males (age: 25 ± 1 years (mean \pm SEM); BMI: 23 ± 1 kg/m²; HbA_{1c}: $5.4 \pm 0.1\%$; no family history of diabetes) before and after intervention using high calorie diet, sedentary lifestyle and administration of prednisolone (37.5 mg/day) for 12 days.

Results: The intervention resulted in insulin resistance according to HOMA (1.2 ± 0.2 vs 2.6 ± 0.5 , $p=0.01$) and glucose tolerance deteriorated as assessed by the AUC for plasma glucose during OGTT (730 ± 30 vs 846 ± 57 mM \times 2h, $p=0.021$). While the amount of i.v. glucose needed to maintain the hyperglycaemic clamp during continuous saline infusion was unchanged ($p=0.738$), the amounts needed to maintain the clamps with GIP (125 ± 8 vs 93 ± 8 g; $p=0.008$) and GLP-1 infusions (146 ± 9 vs 85 ± 11 g; $p=0.0005$), respectively, were significantly lower following intervention. The subjects compensated for the induced IR by significantly increasing their post intervention insulin responses during saline infusion by 2.9 ± 0.5 fold; thus, disposition index was unchanged. In contrast, the insulin responses to GIP or GLP-1 were only weakly up-regulated (1.78 ± 0.3 and 1.38 ± 0.3 fold, $p=0.001$ vs saline). While C-peptide AUCs during the hyperglycaemic clamp were augmented 2.5 and 2.3 fold by GLP-1 and GIP before, the fold augmentation fell to 1.5 and 1.6 after the intervention ($p=0.003$ and 0.04).

Conclusion: These data show that impairment of the insulinotropic effect of both GIP and GLP-1 can be induced in healthy male subjects without risk factors for type 2 diabetes indicating that the reduced insulinotropic effect of the incretin hormones observed in type 2 diabetes most likely is a consequence of insulin resistance and glucose intolerance rather than a primary event causing type 2 diabetes.

Clinical Trial Registration Number: NCT01173978

Supported by: EFSD/Novartis grant

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Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in man

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Background and aims: Longstanding knowledge positions glucose-dependent insulinotropic polypeptide (GIP) as an incretin hormone in healthy humans, but controversy exists regarding the glucagonotropic effects of GIP. We hypothesized that the glucagonotropic effect of GIP, like its insulinotropic effect, is glucose-dependent. Therefore, we aimed to evaluate the effect of GIP on plasma concentrations of glucagon at three different glycaemic levels.

Research design and methods: Ten healthy male subjects (age: 23 ± 1 (mean \pm SEM) years; BMI: 22 ± 1 kg/m²; HbA_{1c}: $5.5 \pm 0.1\%$, no family history of diabetes) were studied on six separate days. Physiological doses of GIP or saline were administered intravenously (randomized and double-blinded) during 90 min of insulin-induced hypoglycaemia, euglycaemia or hyperglycaemia, respectively (randomized).

Results: During hypoglycaemia plasma glucose (PG) was gradually lowered from mean fasting level of 5.0 ± 0.1 mM (mean \pm SEM) to a plateau level of 2.8 ± 0.1 mmol/l. GIP infusion resulted in greater a glucagon response during the first 30 minutes compared to saline (AUC: 76 ± 17 vs 28 ± 16 pmol/l \times 30 min, $p<0.008$) and a small increment in insulin secretion rate (ISR) before PG was lowered (0–10 min). During euglycaemia with a mean PG of 5.0 ± 0.1 mmol/l, GIP infusion elicited larger glucagon responses (62 ± 18 vs -11 ± 8 pmol/l \times 90 min, $p<0.005$) and increased ISR only during the first 5 minutes compared to saline. During hyperglycaemia with a mean PG of 12.1 ± 0.3 mmol/l comparable suppression of plasma glucagon (-461 ± 81 vs -371 ± 50 pmol/l \times 90 min, $p=0.26$) was observed with GIP and saline infusions, whereas GIP infusion more than doubled ISR ($p<0.0001$).

Conclusion: GIP has no effect on glucagon responses during hyperglycaemia when it potentiates insulin secretion. In contrast, GIP increases glucagon levels during fasting and hypoglycaemic conditions, when it has little or no effect on insulin secretion. Thus, GIP seems to be a bifunctional physiological blood glucose stabilizer with diverging glucose-dependent effects on the two main pancreatic glucoregulatory hormones insulin and glucagon.

Clinical Trial Registration Number: NCT01048268

Supported by: Novo Nordisk Foundation

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Two novel peptide analogues of glucagon show glucagon receptor antagonist activity *in vitro* as well as antidiabetic actions in obese diabetic ob/ob mice

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Background and aims: In addition to defective insulin secretion and insulin resistance, abnormal glucagon regulation leading to hyperglucagonaemia, contributes to the hyperglycaemia observed in type 2 diabetes. Here we examined the actions of two potential glucagon receptor antagonist peptides at blocking glucagon induced insulin secretion *in vitro* in clonal pancreatic BRIN-BD11 cells. We also examined their *in vivo* ability to improve glycaemic control in chronically treated hyperglycaemic obese diabetic ob/ob mice.

Materials and methods: The acute (20 min) dose dependent effects of glucagon, or desHis¹Pro⁴Glu⁹-glucagon amide, or its acylated analogue desHis¹Pro⁴Glu⁹Lys¹²glutamyl-PAL-glucagon amide (10^{-12} to 10^{-6} mol/l) were assessed in BRIN-BD11 cells cultured in 5.6 mmol/l glucose. To assess antagonist activity *in vitro* the actions of both analogues (10^{-12} to 10^{-6} mol/l) were tested on insulin secretion in these cells alone or in the presence of a fixed glucagon (10^{-7} mol/l) concentration. For *in vivo* studies three groups of mice ($n=8$, aged 12–18 weeks) were given twice daily i.p. injections (09:30 and 16:30 h) for 10 days, of either desHis¹Pro⁴Glu⁹-glucagon amide, or its acylated analogue desHis¹Pro⁴Glu⁹Lys¹²glutamyl-PAL-glucagon amide (25 nmol/kg) or saline control. Body weight, food intake, non-fasting blood glucose and plasma insulin were monitored at regular intervals. After 10 days of treatment, a glucose tolerance test was performed by oral gavage (18 mmol/l glucose) and the responses to acute i.p. glucagon challenge was assessed.

Results: BRIN-BD11 cell studies showed that glucagon stimulated insulin secretion in a dose-dependent manner ($P<0.05$ to $P<0.001$) showing a 1.9-fold increase at 10^{-6} mol/l glucagon versus 5.6 mmol/l glucose control. In contrast, neither of the glucagon peptide analogues alone (10^{-12} to 10^{-6} mol/l) stimulated insulin secretion in BRIN-BD11 cells, but both dose-dependently inhibited the insulinotropic action of glucagon (10^{-7} mol/l) working best at higher concentrations ($P<0.001$). In mice studies, no differences in body weight or food intake were observed following 10 days of treatment. However, non-fasting blood glucose was reduced (ANOVA, $P<0.01$) on days 5 and 10 and plasma insulin elevated ($P<0.05$ to $P<0.01$) on days 3, 5 and 10 in desHis¹Pro⁴Glu⁹Lys¹²glutamyl-PAL-glucagon treated ob/ob mice. No differences were observed in non-fasting blood glucose or plasma insulin between the desHis¹Pro⁴Glu⁹-glucagon and saline treated mice. Following the 10 day treatment period, oral glucose tolerance was improved in the acylated analogue group ($P<0.05$) but not in the desHis¹Pro⁴Glu⁹-glucagon treated group. Notably when glucagon (25 nmol/kg) was administered to fasted mice 16 h after the normal twice daily peptide treatments (at 08.30 h), an overall blood glucose reduction was observed in both peptide analogue treated groups versus saline groups (AUC, 0–60 min, $P<0.05$).

Conclusion: DesHis¹Pro⁴Glu⁹-glucagon amide and its acylated analogue desHis¹Pro⁴Glu⁹Lys¹²glutamyl-PAL-glucagon amide antagonized glucagon induced insulin secretion in cultured BRIN-BD11 cells. *In vivo* studies showed that chronic treatment with both peptide analogues inhibited glucagon induced hyperglycaemia and that desHis¹Pro⁴Glu⁹Lys¹²glutamyl-PAL-glucagon amide was more effective at improving glucose tolerance and alleviating hyperglycaemia in an animal model of type 2 diabetes.

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Prohormone convertase 2 positive enteroendocrine cells are more abundant in patients with type 2 diabetes: a potential source of gut-derived glucagon?

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Background and aims: In a cells, the precursor proglucagon (from the glucagon gene) is processed by prohormone convertase (PC)2 to glucagon, whereas enteroendocrine L cells utilize PC1 in the processing of proglucagon to the glucagon-like peptides 1 and 2 (GLP-1 and GLP-2). Hyperglucagonaemia

following oral glucose in type 2 diabetes mellitus (T2DM) is thought to arise as a consequence of dysfunctional α cells combined with β cell insufficiency. However, in contrast to oral glucose, i.v. glucose does not elicit hypersecretion of glucagon in T2DM. Therefore, we hypothesized that T2DM patients possess the potential to release glucagon directly from the gut.

Materials and methods: Ten male patients with T2DM (age: 51(41–62) years; BMI: 32(28–39) kg/m²; HbA_{1c}: 7.1(5.4–8.7)%) and 10 male healthy control subjects (age: 58(48–67) years; BMI: 31(26–36) kg/m²; HbA_{1c}: 5.5(5.2–6.0)%) underwent a 4h meal test and a jejunoscopy (including jejunal biopsies) on two separate days.

Results: Patients with T2DM exhibited exaggerated postprandial plasma glucose excursions (379±76 (mean±SEM) vs 77±33 mM×4h, $p=0.001$). Postprandial insulin (30±6 vs 27±5 nM×4h, $p=0.7$) and C-peptide responses (175±25 vs 188±24 nM×4h, $p=0.7$) were similar in the two groups, but patients with T2DM exhibited higher postprandial glucagon responses (3.0±0.5 vs 1.9±0.2 nM×4h, $p=0.02$). No differences in glucose-dependent insulinotropic polypeptide (GIP), GLP-1, GLP-2 or peptide YY responses were observed. Similar numbers of endocrine cells (all stained for PC1) from jejunal biopsies were observed in the two groups; including GIP, GLP-1, and GLP-2 positive cells. Significantly more PC2 positive cells were found among T2DM patients (70±8 vs 44±4 cells/mm², $p=0.01$). Similar levels of PC1 and PC2 gene expression were observed in the two groups.

Conclusion: Our results show that a high number of small intestinal endocrine cells in T2DM patients are equipped with PC2, which potentially - through processing of proglucagon to glucagon - contribute to the hyperglucagonaemia of these patients; shifting the 'pancreacentric' view on type 2 diabetic hyperglucagonaemia towards a role for the gut in this pathophysiological trait.

Clinical Trial Registration Number: NCT00639613

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Evaluation of beta cell function in proglucagon-EGFP knock-in mice

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Background and aims: Under normal physiological conditions, glucagon signaling is important for glucose homeostasis. Hyperglucagonemia has been known to be associated with hyperglycemia in diabetic subjects. Glucagon receptor knockout mice and mice treated with glucagon receptor antagonist exhibited improved glucose tolerance and β -cell function because of increase of GLP-1 production in islets. In this study, we investigated the role of glucagon in glucose metabolism by using mice deficient for proglucagon-derived peptides.

Materials and methods: We generated *Gcg*-EGFP (enhanced green fluorescent protein) knock-in (*Gcg*^{EGFP/EGFP}) mice, which cannot produce glucagon, glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2). We evaluated glucose tolerance by OGTT and IVGTT (2g/kg BW), morphological analysis of pancreatic islets, and insulin secretion in isolated islets.

Results: The serum glucose levels were not different between *Gcg*^{+/+} mice and *Gcg*^{EGFP/EGFP} mice in the fasted and fed states. *Gcg*^{EGFP/EGFP} mice showed low insulin levels in the fed state but not in the fasted state. At 15min after the intravenous glucose loading, the serum insulin levels in *Gcg*^{EGFP/EGFP} mice were significantly higher than those in the *Gcg*^{+/+} mice (*Gcg*^{+/+}, 902±132; *Gcg*^{EGFP/EGFP}, 1537±252pg/ml, $p<0.05$). Furthermore, the isolated islets of *Gcg*^{EGFP/EGFP} mice secreted more insulin than that of *Gcg*^{+/+} mice when stimulated by 2.8mM and 16.7mM glucose concentrations. These results indicated that the glucose-stimulated insulin secretion (GSIS) was enhanced in *Gcg*^{EGFP/EGFP} mice more than in *Gcg*^{+/+} mice. Compared to *Gcg*^{+/+} mice, *Gcg*^{EGFP/EGFP} mice displayed α -cell hyperplasia, increased α/β -cell ratio (*Gcg*^{+/+}, 1.04±0.11; *Gcg*^{EGFP/EGFP}, 2.15±0.12, $p<0.001$), and increased islet numbers (*Gcg*^{+/+}, 0.225±0.025; *Gcg*^{EGFP/EGFP}, 1.776±0.151/10⁶ μ m², $p<0.001$). The β -cell area in *Gcg*^{EGFP/EGFP} mice was similar to that in *Gcg*^{+/+} mice. *Gcg*^{EGFP/EGFP} mice showed low glucose levels (*Gcg*^{+/+}, 445±16; *Gcg*^{EGFP/EGFP}, 343±23mg/dl, $p<0.005$) and enhanced insulin levels (*Gcg*^{+/+}, 704±70; *Gcg*^{EGFP/EGFP}, 2610±614pg/ml, $p<0.05$) at 15 min after the oral glucose administration. In addition, the serum GIP levels of *Gcg*^{EGFP/EGFP} mice at 15 min after the oral glucose administration, were increased compared to those of *Gcg*^{+/+} mice (*Gcg*^{+/+}, 544±69; *Gcg*^{EGFP/EGFP}, 930±138 pg/ml, $p<0.05$). The mice were intraperitoneally pretreated with atropine and saline during the OGTT. In the *Gcg*^{EGFP/EGFP} mice, the enhancement of the insulin secretion observed at 15 min after

the oral glucose loading was partially inhibited by pretreatment with atropine (saline, 4623±894; atropine, 1975±387 pg/ml, $p<0.01$).

Conclusion: These results suggest that enhanced GIP secretion, the activation of the vagus nerves and the facilitation of GSIS are involved in increased insulin secretion in the *Gcg*^{EGFP/EGFP} mice. Here, we present the *Gcg*^{EGFP/EGFP} mice as a useful model for investigating the role of proglucagon-derived peptides in glucose homeostasis.

OP 23 Brain metabolism and food intake

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Both ageing and body mass modulate the human brain response to food cues

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Background and aims: Functional magnetic resonance imaging (fMRI) data suggest the corticolimbic responses to food cues, which control eating behaviour, mature from childhood to early adulthood in man. Changes in such higher functioning brain responses observed in obesity and Type 2 diabetes may be implicated in their aetiology. As the risk of these disorders rises as one ages further, we investigated the effect of advancing age in adults on these neural responses.

Materials and methods: 24 (12 male) non-diabetic subjects (mean age 35.9 [range 19.5 - 52.6] years, BMI 24.9±0.8 kg/m²) were studied twice in random order following an overnight fast. Subjects viewed food and non-food related object images during Blood Oxygenation Level Dependent fMRI 20 minutes after either 554 kcal standard meal (fed) or 50 ml water (fasted). Using a 10-point visual analogue scale, meal-induced satiety was measured intermittently post-ingestion, with concurrent blood sampling for circulating glucose and gut peptides. Food cue-induced hunger was also measured during BOLD data collection. Imaging data were analysed using XBAM software to determine associations between brain responses to food cues with age and BMI. Cluster level maps were obtained with a threshold at <0.5 expected type I error cluster per brain and all cluster-level correlation coefficients were >0.5.

Results: Satiety scores were higher after feeding (AUC above baseline 82.2±13.4 vs 7.1±7.2 units-min, $p < 0.001$). Visual food cues elicited higher hunger scores than non-food cues in both fed and fasted states and all hunger scores were greater when fasted than fed (general linear model main effects: food ingestion $p < 0.001$, image viewing $p = 0.002$). Whilst age was correlated with BMI ($r = 0.56$, $p = 0.004$), neither was significantly associated with satiety or hunger ratings.

In both fasted and fed states, age and BMI positively correlated with the effect size of responses to food vs non-food in the right insula; rising BMI was associated with greater precuneus responses; and increasing age with smaller thalamic responses. When fasted, age was negatively correlated with dorsolateral prefrontal cortex (DLPFC) responses and BMI negatively correlated with anterior cingulate responses. After eating, the effect size of activation by food cues was positively correlated in the middle frontal gyrus with age and BMI, ventral striate (age only) and thalamus (BMI only). Negative correlations in the fed state were observed between age and anterior cingulate and precuneus responses, and between both age and BMI with orbitofrontal cortex activity.

Conclusion: Whilst it is established that BMI influences regional brain responses to food cues, age may independently influence such frontocortical higher executive (DLPFC) and limbic reward (thalamus, ventral striate) neural network responses to food that control appetite and eating behaviour. Reduced activation of these pathways may result in reduced control of food intake, which may be involved in the increased risk of obesity with rising age.

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Abrogation of glutamate consumption in the central nervous system modifies brain metabolism and reshapes peripheral energy distribution

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Background and aims: Glucose is the prominent source of energy delivered to the central nervous system (CNS) by peripheral organs. However, within the brain, oxidative catabolism of the neurotransmitter glutamate is suspected to contribute to maintenance of energy homeostasis. Upon glutamatergic transmission, intersynaptic glutamate clearance is achieved mostly by astrocytes. Following its uptake by astrocytes glutamate may be amidated to glutamine and then recycled back to neurons. Alternatively, glutamate may be deaminated to alpha-ketoglutarate via the mitochondrial enzyme glutamate dehydrogenase (GDH) before further oxidation in the TCA cycle, thereby promoting ATP generation. Here, we generated transgenic mice with brain-specific deletion of GDH, named CnsGlud1^{-/-} to question the importance of GDH as a key enzyme connecting glucose and glutamate metabolism within the CNS.

Materials and methods: GDH deletion in the CNS was assessed by western blot and by enzymatic activity. Mitochondrial oxygen consumption and cytosolic ATP levels were measured in isolated brain tissues. Glucose uptake was estimated by incorporation of injected 2-deoxy-D-[¹⁴C]glucose. Brain metabolites were measured by NMR in living animals, otherwise on isolated tissues using commercial kits. The excitatory transmission was analyzed by electrophysiology. The study has been carried out along the "Principles of laboratory animal care".

Results: Glutamate oxidation was inhibited in knockout brains, correlating with elevated intra-cellular glutamate plus glutamine pool and impaired glutamate-induced ATP generation. This was compensated by increased brain glucose consumption. Consequently, central glucose concentrations were lower in CnsGlud1^{-/-} mice (-47%, $p < 0.05$), while systemic glucose levels were similar to controls. The lack of glutamate oxidation in CnsGlud1^{-/-} brains enhanced brain glucose uptake (+32%, $p < 0.05$) and promoted enhanced liver gluconeogenesis. Redirection of energy substrates to the CNS resulted in lower body weights of CnsGlud1^{-/-} mice, correlating with muscle autophagy and elevated plasma corticosteroid levels (+82%, $p < 0.01$). Fat turnover was modified with increased circulating free fatty acids and ketone bodies as well as enhanced metabolism of peripheral adipose tissue. CnsGlud1^{-/-} mice were glucose intolerant, partly due to lower glucose utilization rate. Electrophysiological studies showed that the phenotype was not associated with any alterations in excitatory transmission in the CNS.

Conclusion: Impairment of glutamate oxidation in the CNS induced by brain-specific GDH deletion led to central hypoglycaemia mimicking severe fasting conditions, in turn mobilizing energy substrates from the periphery.

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Impact of type 2 diabetes on hunger and brain responses to eating: a continuous arterial spin labelling functional magnetic resonance imaging study

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Background and aims: Central control of appetite is an attractive target for weight reduction and diabetes control. Continuous arterial spin labelling (cASL) functional magnetic resonance imaging (fMRI) of the brain quantifies changes in regional cerebral blood flow (rCBF), a surrogate marker of neuronal activation, and has been applied to examine the effect of type 2 diabetes (T2DM) on neuronal responses to feeding.

Methods: 11 right (R) handed people (7 male) with T2DM on

lifestyle±metformin (age 54.7±7.0 yr; BMI 31.1±3.7 kg/m²) and 12 (7 male) right-handed non-diabetic control (age 45.3±4.8 yr; BMI 27.1±3.7 kg/m²) were scanned in a 1.5 Tesla MR Scanner after an overnight fast. Baseline cASL scan was acquired (~14 min), subjects consumed either a 554 kcal mixed meal (fed) or 50mls water (fasted) in random order (~6 min) and 3 further cASL scans were acquired at 0, 8 and 28 min. Appetite was measured using visual analogue scale (VAS) with concurrent blood sampling at -15, -7, 7, 15, 27 and 35 min. VAS data were analysed using SPSS and a flexible factorial design analysis in Statistical Parametric Mapping 5 was applied to cASL data to examine the effect of T2DM on rCBF changes to food. All clusters were at a corrected cluster-level p-value< 0.05.

Results: Hunger scores were lower in T2DM ($p<0.01$), but did not fall in the fed state, in contrast to the controls, where scores of the range 1 to 10 fell from 6.25±1.05 to 5.00±0.87. Preliminary analysis of the regional responses, corrected for the global differences, in the T2DM group during fasting, compared to controls, showed greater rCBF in R-hypothalamus, R-thalamus, R-medial globus pallidus, L-posterior cingulate, R-lingual gyrus and bilateral(B)-pons and lower rCBF in R-dorsolateral prefrontal gyrus (DLPFC), R-insula, R-frontal gyrus, B-precuneus, and R-superior occipital gyrus. After eating, the T2DM group showed greater rCBF in R-inferior temporal gyrus, fusiform gyri and parahippocampal gyri and lower rCBF in R-medial frontal gyrus, L-DLPFC, B-precuneus and cingulate gyri, compared to controls.

Conclusion: During fasting, T2DM subjects exhibited lower hunger; with greater neuronal activation in brain regions associated with homeostasis and gustatory system, and reduced responses in neural networks associated with reward and satiation modulation. In response to a small meal, T2DM subjects had less relief of hunger and less neuronal activation in reward networks, with greater activation in regions associated with hunger. Altered central satiety signalling in T2DM may be a barrier to weight reduction and lifestyle management in the treatment of T2DM.

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GLP-1 differentially affects blood-brain glucose transfer and metabolism in humans

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Background and aims: Glucagon-like peptide-1 (GLP-1) has pronounced effects on glucose metabolism, and results in cells and intact animals suggest a protective effect on brain tissue. The mechanism is unknown. Glucose transport across the blood-brain barrier (BBB) has few known regulatory mechanisms other than the arterial glucose concentration and the cerebral capillary density or capillary surface area. We recently reported reduction of blood-brain glucose transfer with elevated GLP-1 in humans in whom glucose and insulin concentration were maintained at constant levels. This change could be due to changes of maximum glucose transport capacity or glucose transporter affinity or both. Thus, the working hypothesis claims that GLP-1 influences the transport of glucose across the BBB.

Materials and methods: To test this claim, we determined blood-brain glucose transfer and brain glucose metabolic rate by means of PET of FDG uptake at low and high glucose concentrations with and without GLP-1 and correction for lumped constant changes. With the PET, we also determined the blood-brain clearance of glucose, intracerebral glucose concentration and phosphorylation rate of hexokinase.

Results: At low plasma glucose levels, GLP-1 did not change cerebral glucose uptake, net clearance, cerebral metabolic rate, or phosphorylation rate. In hyperglycemia, GLP-1 increased the net clearance of glucose by 5–11% ($P=0.046$) in individual grey and white matter regions. In thalamus, the metabolic rate of glucose increased by 8% ($P=0.03$), with a similar trend throughout the brain. The same regions revealed a uniform trend toward increased phosphorylation rate (8–14%, $P=0.08$), significantly so in thalamus ($P=0.04$), and decreased intracerebral glucose concentrations (5–8%, $P=0.12$). Determined from low and high glucose levels together, GLP-1 affected maximum BBB glucose transport capacity in striatum by raising the T_{max} significantly from 1.1 to 1.3 μmol/g/min (18%, $P=0.03$) with a similar trend in the other regions, and the transporter affinity K_t insignificantly from 5 to 7 mM. The data reveals the same trend towards increased glucose metabolism by raising the maximum phosphorylation rate V_{max} from 0.29 to 0.31 μmol/g/min, and the hexokinase affinity K_m from 0.01 mM to 0.08 mM (means of regions).

Conclusion: These preliminary results suggest that GLP-1 increases glucose clearance in the brain and significantly changes glucose transport capacity

in striatum, with a uniform trend for the grey matter as a whole. Thus, the metabolic rate increased and the cerebral tissue glucose concentration decreased, together with a trend towards increased phosphorylation rates and decreased affinity of hexokinase towards glucose. These changes appear to be opposite the changes reported by Gjedde and Crone in chronic hyperglycemia of experimentally diabetic rats in which T_{max} declined significantly and K_t tended to decline but did not reach significance.

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The brain's response to food ingestion after Roux-en-Y gastric bypass: a [¹⁸F]-fluorodeoxyglucose positron emission tomography (FDG-PET) study

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Background: The mechanisms whereby Roux-en-Y gastric bypass (RYGB) causes weight loss and improved glycaemic control in Type 2 diabetes are not fully understood. One possibility is that changes in gut to brain communication may modulate central control of appetite. Anecdotally people who have had RYGB report increased fullness when eating. **Aim:** To investigate the brain's response to food ingestion in people who have lost weight post RYGB using [¹⁸F]-fluorodeoxyglucose positron emission tomography (FDG-PET) functional neuroimaging.

Materials and methods: Four groups of people have been studied: four individuals 27±12months post RYGB (BMI 36.6±3.0kg/m², weight loss 32.7±12.1% pre-surgical weight, HOMA2IR 0.55 ± 0.10); seven non-operated obese insulin sensitive, ObIS (BMI 34.6±2.4kg/m², HOMA2IR 0.54±0.11); seven non-operated obese insulin resistant, ObIR (BMI 33.5±1.9kg/m², HOMA2-IR 1.83±0.64); and eight normal weight, NW (BMI 22.3±1.2kg/m², HOMA2-IR 0.55±0.17). Individuals underwent FDG-PET brain imaging on 2 occasions in random order after an overnight fast: once FED (400kcal mixed meal consumed 15mins prior to FDG injection) and once FASTED (no calorie intake). Brain FDG uptake, a marker of neuronal activation, was compared using Statistical Parametric Mapping. Satiety was assessed using visual analogue scales and post-scan ad-libitum meal.

Results: For all groups combined, satiety scores increased at the 10minutes post meal timepoint in the FED state ($p<0.001$) with no difference between the four groups (rmANOVA, $p=0.365$). For all groups combined, the FED state was associated with reduced food consumption in the post scan ad-libitum meal (mean decrease 147±43kcal, $p=0.002$) with no difference in the decrease between the four groups. The post RYGB group consumed significantly less in the ad-libitum meal at the FASTED visit than the other three groups (ANOVA, $p=0.016$). Preliminary analysis shows that in the post RYGB group the FED, compared to the FASTED, state was associated with significant decreases (cluster-level corrected $p<0.05$) in FDG uptake in precuneus, areas of the prefrontal cortex (PFC) including the dorsolateral prefrontal cortex (DLPFC) and the orbitofrontal cortex and in the thalamus. Relative deactivation after food was also seen in precuneus and areas of the PFC, including the DLPFC, in NW. In contrast, there were no areas of decreased neuronal activation in the FED state in either of the non-operated obese groups. The extensive neuronal activation in the FED state in the ObIR group was not seen post RYGB.

Conclusion: The pattern of central neuronal deactivation in response to food ingestion in people who have had RYGB is similar to normal weight subjects' responses and different from those of obese subjects who have not had surgery. These changes are compatible with an effect of RYGB surgery on central responses to food ingestion which may explain increased satiation with eating and the weight loss seen post RYGB.

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It's between the ears: role of hypoxia inducible factor 1alpha (HIF1alpha) activity in obesity

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Background and aims: We previously reported that the transcription factor HIF1alpha is down regulated by 90% in islets from people with type 2 diabetes. Mice lacking beta-cell HIF1alpha have mild glucose intolerance. Mice on a high fat diet (HFD) plus iron chelator (IC), to increase protein levels of HIF1alpha, had improved glucose tolerance and beta-cell function. An unexpected finding was that mice on a HFD+IC had significantly reduced weight gain. The hypothalamus regulates energy expenditure, body temperature and food intake. We hypothesised that decreased obesity was due to hypothalamic activation of HIF1alpha. To test this hypothesis, we have made hypothalamic HIF1alpha knockout mice.

Materials and methods: Using floxed HIF1alpha mice and adenovirus expressing Cre-recombinase, we generated mice with a hypothalamic-specific deletion of HIF1alpha by direct bilateral stereotaxic injection into the arcuate nuclei in the hypothalamus. Mice were given HFD±IC to increase HIF1alpha. Mice underwent DEXA body composition scanning, Oxymax respiratory rate- and food intake measurements.

Results: Hypothalamic HIF1alpha deletion blocked ~50% of the weight-protection effect of IC in HFD fed mice ($p<0.01$). Food intake and brown adipose tissue weights were increased in both control and hypothalamic HIF1alpha knockout HFD+IC mice, but hypothalamic HIF1alpha knockout mice gained a greater amount of weight.

Conclusion: This data demonstrates a novel role for hypothalamic HIF1alpha activity in control of weight and metabolic rate. Arcuate nucleus HIF1alpha is necessary for the full weight-protection effect of IC in HFD fed mice demonstrating that a substantial part of the effect is via hypothalamic HIF1alpha. These studies identify a novel mechanism of weight regulation.

OP 24 Beta cell damage

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Beta cell dysfunction and loss induced by ablation of mitochondrial chaperone prohibitin-2 in pancreatic beta cells promotes diabetes in transgenic Bet-Phb2^{-/-} mice

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Background and aims: Prohibitin-1 and -2 are homologues of prohibitin family implicated in cell cycle, ROS production, cancer, obesity, and inflammation. They form ring-shaped complexes in the inner mitochondrial membrane, controlling cristae remodeling and apoptosis. In pancreatic beta cells, mitochondria play pivotal role in metabolism-secretion coupling. Nevertheless, there is paucity of information regarding mitochondrial dynamics in general and putative role of prohibitins in particular. We generated β -cell specific Phb-2 knockout mice (Bet-Phb2^{-/-}) to study prohibitins and mitochondrial integrity in the regulation of glucose homeostasis, beta cell physiology and survival. **Materials and methods:** Bet-Phb2^{-/-} mice were generated by mating mice with Phb2 alleles flanked by lox-P sites (Phb2^{fl/fl}) with animals carrying Cre-recombinase driven by the rat insulin promoter. Phb2 deletion was confirmed by genomic PCR and immunoblotting on isolated tissue extracts. *In vivo* glucose homeostasis was characterized by glucose tolerance test and measurement of plasma insulin levels by ELISA. We studied glucose-stimulated insulin secretion (GSIS) by *in-situ* pancreatic perfusions. Glucose-stimulated changes in insulin release, mitochondrial membrane potential and cytosolic calcium concentrations were measured in isolated islets. mtDNA copy number was analyzed by qPCR. Islet morphology, β - and α -cell mass, proliferation and beta cell loss were studied using immuno-histochemistry. The study has been carried out along the "Principles of laboratory animal care".

Results: Bet-Phb2^{-/-} mice which were normoglycaemic until the age of 4 weeks became hyperglycaemic at 6 weeks (average glycaemia 14.6 mM, $p<0.05$), and thereafter exhibited severe diabetes. At 6 weeks, Bet-Phb2^{-/-} mice were glucose intolerant (ipGTT: AUC +133%, $p<0.001$) with lower fasting plasma insulin levels (-66%, $p<0.01$) compared to littermate control (Phb2^{fl/fl}) mice. *In-situ* pancreatic perfusions revealed that both phases of GSIS were markedly reduced in Bet-Phb2^{-/-} mice (-75%, $p<0.001$). Compared to Phb2^{fl/fl} islets, those isolated from 4-week old Bet-Phb2^{-/-} mice were poorly responsive to glucose stimulation regarding insulin secretion, mitochondrial membrane potential, ATP generation, and cytosolic calcium rise. In addition, mtDNA copy number was reduced by 50% in Bet-Phb2^{-/-} islets. Strikingly, β -cell proliferation was 2.5-fold higher in Bet-Phb2^{-/-} than controls at the age of 4 weeks. However, beta cell mass progressively declined with age in Bet-Phb2^{-/-} mice, showing 35% and 90% reduction at 4 and 10 weeks of age respectively, accompanied by remodeling of islet architecture. On the contrary, α -cell mass was increased at 10 weeks in Bet-Phb2^{-/-} compared with control littermates.

Conclusion: This study demonstrates that impairment in mitochondrial integrity per se, i.e. not induced by toxic agents, secondary to deletion of prohibitin-2 results in β -cell dysfunction and gradual loss promoting diabetes. This emphasizes the importance of PHB2 in beta cell physiology and survival. Interestingly, β -cell proliferation compensated beta cell loss and postponed diabetes during pre-adult stage, whereas loss of β -cells at a later stage of life enlarged α cell mass.

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Survival of rat pancreatic islets is partly controlled by a TCF7L2-p53-p53INP1 dependent pathway

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Background and aims: TCF7L2 is both an activator and an inhibitor of transcription and the most highly associated type 2 diabetes gene known to date.

It influences beta cell survival and function, i.e. incretin hormonal effects, insulin processing and secretion. However, its target genes in pancreatic islets are not fully described and the molecular mechanism whereby it propagates its effects on islet function is not known. The aim of this study is to identify the molecular mechanisms through which TCF7L2 influence beta cell survival and function.

Materials and methods: Wistar rat primary islets and INS-1 (832/13) cells were incubated with siRNA against Tcf7l2, both Tcf7l2 and TP53INP1 or both TCF7L2 and TP53 in 5.5 mM and 14.3 mM glucose. TCF7L2 activity, p53 activity and target gene expression (using qPCR) were measured after siRNA treatment. INS-1 cell apoptosis was measured by DNA degradation levels, caspase-3/7 levels and by using antibodies against Annexin V, and 7-AAD, visualized using confocal microscopy. Rat islet viability was estimated measuring metabolic rate. Rat islet apoptosis was estimated by measuring Caspase-3/7 level.

Results: The type 2 diabetes associated genes TP53INP1, FTO, GIPR and ADAMTS9 were identified as TCF7L2 potential target gene using chromatin immunoprecipitation on microarrays. In INS-1 cells, siRNA mediated Tcf7l2 knock down (69.5 %) resulted in decreased TCF7L2 activity (91%) and differential expression of the target genes: Tp53 (14.5% increase), TP53INP1 (65.9% increase) and ADAMTS9 (82.8% decrease). TCF7L2 knockdown also lead to reduced cell viability (65%) and increased apoptosis (113%). The TCF7L2 induced cell death was replicated in rat primary islets. When restoring (decreasing) the Tp53inp1 expression level in TCF7L2 depleted islets, the decrease in cell viability and increase in apoptosis were prevented, suggesting that the Tcf7l2 effect is mediated via Tp53inp1. Furthermore, p53 depletion prohibited TCF7L2 down regulation induced cell death and elevation of Tp53inp1 expression in both INS-1 cells and rat primary islets.

Conclusion: The type 2 diabetes associated genes TP53INP1 and ADAMTS9 are target genes of TCF7L2 in pancreatic islets. TCF7L2 induced apoptosis and decreased cell viability are mediated through activation of p53 and increased p53INP1 expression.

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Pancreatic beta cells activate a JunB/ATF3-dependent survival pathway during inflammation

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Background and aims: Infiltrating immune cells contribute to beta cell apoptosis in type 1 diabetes (T1D) at least in part via release of the pro-inflammatory cytokines IL-1 β , IFN- γ and TNF- α . These cytokines activate several downstream transcription factors, including STAT1, NF- κ B and Activator Protein-1 (AP-1). While the role of STAT1 and NF- κ B in beta cell apoptosis has been studied in detail, the role of AP-1 members is less known. We therefore aimed to elucidate the contribution of JunB/AP-1 in cytokine-induced beta cell apoptosis.

Materials and methods: The experiments were performed in human islets from non-diabetic organ donors, FACS-purified primary rat beta cells, insulin-producing INS-1E cells, islets from Ubi-JunB transgenic mice or wild type littermates, and pancreas from pre-diabetic NOD or control mice. Islets, INS-1E cells or rat beta cells were cultured in the presence or absence of cytokines (TNF- α + IFN- γ) and microarray analysis, real time RT-PCR, Western blot, immunohistochemistry, small interfering RNA (siRNA), chromatin immunoprecipitation, adenoviral vector infection, promoter reporter assays and cell viability assays (Hoeschst 33342/propidium iodide staining) were used to clarify the gene networks involved in the cytokine-induced JunB/AP-1 pathway in beta cells.

Results: The cytokine combination TNF- α + IFN- γ up-regulated the expression of the AP-1 component JunB in human islets at both mRNA (2.8 fold increase relative to the controls, n=6, P<0.001) and protein levels. Similar results were observed in primary rat beta cells and clonal INS-1E cells. Increased JunB expression was confirmed by immunohistochemistry in pancreatic islets of pre-diabetic NOD mice. JunB knockdown (KD) by RNA interference (>60% KD) exacerbated cytokine induced beta cell death in primary rat beta cells and INS-1E cells (56–62% increase compared to controls, n=3–5, P<0.05). The gene networks affected by JunB were studied by microarray analysis of INS-1E cells exposed to TNF- α + IFN- γ with or without simultaneous JunB KD by siRNA. We observed that JunB regulates 20–25% of the cytokine-modified beta cell genes including the transcription factor ATF3. ATF3 expression was increased in cytokine-treated human islets (6.2 fold induction compared

to controls, n=6, P<0.01) and *in vitro* JunB silencing led to >60% reduction in ATF3 overexpression. We confirmed direct JunB regulation of the ATF3 promoter activity by its binding to an ATF/CRE site. Importantly, silencing ATF3 aggravated TNF- α + IFN- γ -induced cell death in dispersed human islets (28% increase versus controls, n= 4, P<0.02). Genetic overexpression of JunB in the Ubi-JunB transgenic mouse model increased ATF3 expression in the pancreatic islets and reversed the pro-apoptotic effects of cytokines on beta cells (47 % protection, n=4, P<0.001).

Conclusion: The AP-1 component JunB regulates a complex gene network in differentiated beta cells, leading to improved beta cell survival during exposure to pro-inflammatory cytokines. A key pro-survival gene induced by JunB is ATF3, and cytokine induction of the JunB/ATF3 pathway in beta cells may be critical for preserving beta cell viability in early T1D.

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Exendin-4 protects beta cells from palmitate-induced apoptosis by reducing GPR40 expression and interfering with activation of the MKK4/7-JNK pathway

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Background and aims: Free fatty acids (FFA) induce beta cell damage when their levels are chronically increased, thus contributing to the pathogenesis of type 2 diabetes. GLP-1 and its receptor agonist exendin-4 (ex-4) increase the survival of beta cells exposed to various pro-apoptotic stimuli, including FFA. The aim of this study was to investigate the mechanisms of the protective effects of GLP-1 mimetics on FFA-induced beta cell apoptosis.

Materials and methods: Isolated human islets and rat insulin-secreting INS-1 cells were incubated with 10 nM ex-4 for 16 h in the presence or absence of 0.5 mM palmitate. Protein content and phosphorylation of intracellular signaling intermediates were evaluated by immunoblotting and immunofluorescence techniques. Gene expression was evaluated by qRT-PCR. Silencer pre-designed siRNAs and silencer non-targeting siRNAs (negative control) for IB1 were used. Beta-cell apoptosis was quantified by an ELISA assay evaluating oligosome release into the cytosol.

Results: Exposure of human and rat beta-cells to 0.5 mM palmitate induced a 2.5-fold increase in cell apoptosis measured by evaluation of cytosolic oligosomes and cleaved caspase-3 (p<0.05). Palmitate increased the phosphorylation levels of both JNK and p38 (3.5-fold and 2-fold, respectively; p<0.05) evaluated both by immunoblotting and immunofluorescence. Inhibition of JNK (using the JNK inhibitors SP600125 and JNKi) or p38 (using the p38 inhibitor SB203580) phosphorylation prevented/reduced palmitate-induced apoptosis, respectively (p<0.05). Treatment with 10 nM ex-4 inhibited JNK and reduced p38 phosphorylation levels, and prevented apoptosis in response to palmitate (p<0.05). Furthermore, ex-4 inhibited palmitate-induced phosphorylation of the upstream kinases MKK4 and MKK7, which are implicated in JNK and p38 activation (p<0.05), and increased the protein content of Islet-Brain 1 (IB1), an endogenous JNK blocker (p<0.05). However, RNAi-mediated suppression of IB1 protein levels did not impair the ability of ex-4 to inhibit JNK and to prevent apoptosis. Finally, ex-4 reduced the mRNA levels of GPR40, a cell-surface FFA transporter (p<0.05). The inhibitory effects of ex-4 on JNK and GPR40 were abrogated in the presence of the PKA inhibitor H89.

Conclusion: The activation of the stress-kinases JNK and p38 is involved in palmitate-induced apoptosis in rat and human beta cells. The GLP-1 analog ex-4 counteracts the palmitate effects by reducing GPR40 gene expression and inhibiting MKK7- and MKK4-dependent phosphorylation of JNK and p38 in a PKA-dependent manner.

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The mitochondrial Atp8 mutation induces mitochondrial ROS generation, secretory dysfunction and beta cell mass adaptation in conplastic B6mt^{FVB} mice

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Background and aims: The conplastic mouse strain B6mt^{FVB} carries a mitochondrial DNA mutation in the Atp8 gene coding for the regulatory subunit 8 of ATP-synthase. In comparison to B6mt^{AKR} mice the B6mt^{FVB} strain showed elevated H₂O₂ production in isolated mitochondria. It was the aim of the study to investigate the effect of the Atp8 mutation (1) on the mitochondrial ROS production and secretory function in beta cells under conditions of glucolipotoxicity, and (2) on the metabolic profile under conditions of a high fat diet in vivo.

Materials and methods: Isolated pancreatic islet cells from B6mt^{AKR} (AKR) and B6mt^{FVB} (FVB) mice were exposed to 5 or 30 mM glucose in the presence or absence of 500 μ M palmitic acid. Mitochondrial ROS production was quantified by fluorescence microscopy after Mitosox loading of islet cells. B6mt^{AKR} and B6mt^{FVB} mice were fed high fat diet (HFD, 34% fat) or control diet (CD, 4% fat) for 26 weeks directly after weaning. Body weight, blood glucose, serum insulin, i.p. glucose tolerance, i.p. insulin sensitivity and beta cell mass were monitored at different time points of the diet.

Results: At 5 mM glucose islet cells from B6mt^{FVB} mice showed a 3-fold higher mitochondrial ROS production than islet cells from B6mt^{AKR} (24 h incubation, ratio Mitosox/Dapi: 0.9 ± 0.1 vs. 3.2 ± 0.2 ; $n = 6-8$; $p < 0.05$). Incubation of islet cells at 30 mM glucose resulted only in a minor increase in ROS production in both strains whereas ATP levels were significantly lower in beta cells from the FVB strain. Islets from FVB mice showed a more pronounced impairment of glucose stimulated insulin secretion (GSIS) after incubation with 500 μ M palmitic acid at high and low glucose concentrations. GSIS was significantly reduced in islets from FVB animals after incubation with 30 mM glucose for 24 hours. Feeding high fat diet for 26 weeks resulted in a more pronounced gain of body weight in the FVB strain with a gender dimorphism in favour of female mice. After 18 weeks of high fat diet FVB mice showed significantly elevated blood glucose levels (9.0 vs. 8.2 mM, $p < 0.05$) and an impaired glucose tolerance in comparison to AKR control mice (AUC: 1633 ± 104 vs. 1481 ± 60 ; $p < 0.05$). Serum insulin levels were significantly reduced in FVB mice reflecting secretory dysfunction after HFD. Insulin sensitivity of FVB and AKR mice was comparable after 6 month of HFD. Beta cell mass was increased in FVB mice after HFD by 92% while AKR mice showed a reduction of 18%.

Conclusion: The mutation of the Atp8 subunit of the ATP synthase induced an increased mitochondrial ROS production in isolated pancreatic beta cells at physiological glucose concentrations. However, mitochondrial dysfunction rather than oxidative damage appeared to be crucial for loss of secretory responsiveness under conditions of metabolic stress. Notably, high fat diet induced an adaptive increase of beta cell mass in FVB mice. The data implicate a dual role of mitochondrial ROS for regulation of beta cell mass and dysfunction.

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Could miRNAs derived from lymphocytes trigger beta cell death in type 1 diabetes?C. Guay¹, E. Roggli¹, C. Briet², V. Menoud¹, S. Gattesco¹, C. Boitard², R. Regazzi¹;¹DBCM, University of Lausanne, Switzerland, ²Hôpital Cochin/St Vincent de Paul, INSERM U986, Paris, France.

Background and aims: During type 1 diabetes development, lymphocytes infiltrate pancreatic islets, resulting in beta-cell dysfunction and death. These events are thought to be mediated by proinflammatory cytokines. However, immune cells release also microvesicles, like exosomes, containing mRNAs, microRNAs and proteins that can be transferred in active form to recipient cells. Since during insulinitis beta cells and T cells are brought in close proximity and microRNAs are important regulators of beta cell functions, we hypothesized that exosome-mediated transfer of specific microRNAs from T cells to pancreatic beta cells could contribute to the dysfunction of insulin-secreting cells in the early phases of type 1 diabetes.

Materials and methods: To test this novel concept, exosomes were isolated by ultracentrifugation from culture media of the Jurkat T cell line or from

primary T effector cells of NOD mice. These microvesicle preparations were analyzed to determine their microRNA content and were used to study microRNA transfer from T cells to the beta cell line MIN6 or to dispersed primary rat islet cells.

Results: We first observed that five microRNAs, miR-142-3p, miR-142-5p, miR-150, miR-155 and miR-216, are highly upregulated in pancreatic islets during insulinitis in pre-diabetic NOD mice. Except for miR-216, these microRNAs are over a thousand times more abundant in T cells compared to beta-cells. These microRNAs were also found to be released in microvesicles originating from Jurkat T cells and primary T effector cells. When microvesicles of Jurkat T cells were applied to beta-cells, miR-142-3p, miR-142-5p, miR-150 and miR-155 were increased in recipient cells but not miR-216 or miR-29a, a microRNA highly expressed in beta cells. We next investigated the functional effect of Jurkat-derived microvesicles on MIN6 cells and on dispersed primary islet cells. We found that the cells exposed to T cell-derived vesicles display an increase in apoptosis measured by Hoechst and AnnexinV staining and a reduction in insulin secretion in response to glucose. To correlate the increase in microRNA expression to the functional effect observed upon exposure to the microvesicles, we investigated the impact of miR-142-3p and miR-142-5p overexpression on beta-cell functions. Increased miR-142-5p expression in MIN6 cells or dispersed islet cells promoted cell death while miR-142-3p overexpression reduced glucose-induced insulin secretion.

Conclusion: Taken together, our results suggest that microRNAs associated with microvesicles released by T cells can be picked up in active form by beta cells. The transfer of these regulators of gene expression correlates with alterations of beta cell functions. More experiments will be needed to proof that beta cell dysfunction is due to the microRNAs associated with the microvesicles. However, our results support the concept that microRNA transfer constitute a novel cell-to-cell communication mechanism.

OP 25 SGLT-2 inhibitors

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Dapagliflozin, Metformin-XR, or both together to initiate pharmacologic therapy for type 2 diabetes

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Background and aims: Combining metformin-XR (MET) with another oral antidiabetic agent as initial therapy for T2DM may be advantageous in patients who have high HbA_{1c} with diet and exercise. Dapagliflozin (DAPA) is a selective SGLT2 inhibitor that lowers hyperglycaemia independently of insulin secretion or action by inhibiting renal glucose reabsorption. We report results from 2 randomised, double-blind, active-controlled, 24-week trials of DAPA and MET; combined or alone, in treatment-naïve patients with T2DM inadequately controlled with diet and exercise. Study 1 (MB102021) compared treatment regimens of once daily DAPA 5 mg + MET, DAPA 5 mg, and MET. Study 2 (MB102034) compared regimens of once daily DAPA 10 mg + MET, DAPA 10 mg, and MET.

Materials and methods: Patients with age range 18–77 years and HbA_{1c} 7.5–12% were eligible for inclusion. In each trial MET was titrated up to 2000 mg, with the majority of patients receiving MET 2000 mg daily. The primary endpoint was change from baseline in HbA_{1c} at week 24 (LOCF). Secondary endpoints included changes from baseline in fasting plasma glucose (FPG) and weight at week 24 (LOCF).

Results: In both trials DAPA+MET was more effective than either drug alone in reducing HbA_{1c} and FPG, and more effective than MET in reducing weight (table). In a pre-specified comparison of groups in Study 2, DAPA 10 mg was non-inferior to MET in reducing HbA_{1c}, and was superior in reducing both FPG and weight. Signs and symptoms suggestive of genital infection were reported in 6.7%, 6.9%, 2.0% (Study 1) and 8.5%, 12.8%, 2.4% (Study 2) of patients in the respective DAPA+MET, DAPA and MET arms. Signs and symptoms suggestive of urinary tract infection were reported in 7.7%, 7.9%, 7.5% (Study 1) and 7.6%, 11.0%, 4.3% (Study 2) of patients in the respective DAPA+MET, DAPA and MET arms. No event of major hypoglycaemia was reported in either study.

Conclusion: DAPA+MET used to initiate pharmacologic therapy for T2DM was generally well tolerated and effective in reducing HbA_{1c}, FPG and weight.

Results at Week 24 (LOCF)

	Study 1 (n=598)			Study 2 (n=638)		
Adjusted mean change from baseline (SE)	MET	DAPA 5mg	DAPA 5mg + MET	MET	DAPA 10mg	DAPA 10mg + MET
HbA _{1c} , %	-1.35 (0.09)	-1.19 (0.09)	-2.05 ^a (0.09)	-1.44 (0.08)	-1.45 ^a (0.07)	-1.98 ^a (0.08)
FPG, mmol/L	-1.86 (0.15)	-2.33 (0.15)	-3.39 ^a (0.15)	-1.93 (0.14)	-2.58 ^a (0.14)	-3.35 ^a (0.14)
FPG, mg/dL	-33.6 (2.7)	-42.0 (2.7)	-61.0 ^a (2.8)	-34.8 (2.5)	-46.4 ^a (2.5)	-60.4 ^a (2.5)
Weight, kg	-1.29 (0.24)	-2.61 (0.24)	-2.66 (0.24)	-1.36 (0.24)	-2.73 ^d (0.23)	-3.33 ^d (0.24)
% Patients with therapeutic glycaemic response of HbA _{1c} <7.0%, adjusted for baseline (SE)	35 (3)	23 (3)	52 ^b (4)	35 (3)	32 (3)	47 ^c (3)

Significant at: ^a P<0.0001, ^b P≤0.0003, and ^c P≤0.02 vs both DAPA and MET; ^d P≤0.002 vs MET.

Non-inferior to MET.

Clinical Trial Registration Number: NCT00643851 and NCT00859898

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Sustained efficacy of dapagliflozin when added to metformin in type 2 diabetes inadequately controlled by metformin monotherapy

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Background and aims: The selective SGLT2 inhibitor dapagliflozin (DAPA) reduces hyperglycaemia independently of insulin secretion or action by inhibiting renal glucose reabsorption. This study (MB102014) is a randomised double-blind, placebo (PBO)-controlled trial of DAPA added to metformin (MET) in T2DM (n=546) inadequately controlled with MET alone. Previously reported short-term data at week 24 showed significant mean reductions in the primary [HbA_{1c}] and secondary [fasting plasma glucose (FPG) and weight] endpoints with DAPA compared to PBO. Here we report efficacy and safety results at week 102 of the long-term extension.

Materials and methods: Patients aged 18–77 years with HbA_{1c} 7–10% received DAPA 2.5 mg, 5 mg, 10 mg or PBO, plus open-label MET (≥1500mg/d). Exploratory endpoints at week 102 included changes from baseline in HbA_{1c}, FPG and weight, and were analyzed by longitudinal repeated measures analysis.

Results: Overall 71.2% of patients completed 102 weeks of the study; fewer on PBO (63.5%) than on DAPA 2.5 mg, 5 mg, and 10 mg (68.3%, 73.0%, 79.8%), due mainly to more patients on PBO discontinuing for lack of efficacy. At week 102, all DAPA groups showed greater mean reductions from baseline in HbA_{1c}, FPG and weight compared to PBO (table), effects that were similar to those observed at week 24 and maintained throughout the trial. More patients at week 102 also achieved a therapeutic response of HbA_{1c} <7% with DAPA 2.5 mg, 5 mg, and 10 mg (20.7%, 26.4%, 31.5%) than with PBO (15.4%). Adverse events (AEs), serious AEs and AEs leading to discontinuation were balanced across all groups. Signs and symptoms suggestive of genital infection (GenInf) were reported in 11.7%, 14.6%, 12.6% (DAPA 2.5 mg, 5 mg, 10 mg) and 5.1% (PBO) of patients, with 1 discontinuation due to GenInf. Signs and symptoms suggestive of urinary tract infection (UTI) were reported in 8.0%, 8.8%, 13.3% (DAPA 2.5 mg, 5 mg, 10 mg) and 8.0% (PBO), with 1 discontinuation due to UTI. No event of pyelonephritis was reported.

Conclusion: In comparison to PBO, DAPA added to MET over 102 weeks demonstrated greater and sustained improvements in glycaemic control, clinically meaningful reduction in weight, and no increased risk of hypoglycaemia in patients with T2DM inadequately controlled with MET alone.

Results at Week 102

Adjusted mean change from baseline (SE) ^a	PBO + MET	DAPA 2.5mg + MET	DAPA 5mg + MET	DAPA 10mg + MET
HbA _{1c} , %	0.02 (0.11)	-0.48 (0.10)	-0.58 (0.10)	-0.78 (0.09)
FPG, mmol/L	-0.58 (0.20)	-1.07 (0.18)	-1.47 (0.16)	-1.36 (0.15)
FPG, mg/dL	-10.4 (3.6)	-19.3 (3.2)	-26.5 (2.8)	-24.5 (2.7)
Body weight, kg	-0.7 (0.5)	-2.2 (0.5)	-3.4 (0.4)	-2.8 (0.4)
Percent of patients with ≥1 hypoglycemia event ^b	5.8	3.6	5.1	5.2

^aLongitudinal repeated measures analysis. Excludes data after rescue.

^bIncludes data after rescue.

Clinical Trial Registration Number: NCT00528879

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ENCORE: efficacy and safety of BI 10773, a new sodium glucose co-transporter-2 (SGLT-2) inhibitor, in type 2 diabetes patients inadequately controlled on metformin

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Background and aims: Sodium glucose cotransporter-2 (SGLT-2) inhibition is a novel insulin-independent glucose-lowering target in type 2 diabetes (T2D). The aim of this double-blind, placebo-controlled 12-week study was to assess the efficacy and safety of 5 doses of BI 10773, a potent SGLT-2 inhibitor in T2D patients inadequately controlled on metformin.

Materials and methods: A total of 495 subjects (mean baseline HbA_{1c} 7.9%, age 58 years, male 51%, BMI 31.4 kg/m²) were randomized to BI 10773 (1mg, 5mg, 10mg, 25mg, or 50mg once daily [qd]), or placebo (PBO), or open-label sitagliptin 100mg (SITA), added to metformin, for 12 weeks. Primary endpoint was change in HbA_{1c} from baseline to week 12.

Results: Significant and dose-dependent reductions in HbA_{1c} vs PBO were shown with BI 10773 with a maximum PBO-subtracted lowering of about 0.7% with the 10mg and 25mg doses, whereas SITA lowered HbA_{1c} by about 0.6% vs PBO. Significant reductions in fasting plasma glucose (FPG) and body weight vs PBO were observed in all BI 10773 groups except 1mg. A maximum PBO-corrected reduction in systolic BP of about 6 mmHg was seen with BI 10773 25mg. Frequency of adverse events (AEs) was similar in all groups (BI 10773: 38.5%, PBO: 36.6%, SITA: 35.2%). The most frequently reported AEs in the BI 10773 groups vs PBO were urinary tract infection (UTI) (3.1% vs 2.8%) and pollakiuria (2.5% vs 1.4%). Genital infections were reported on BI 10773 (2.5%) and SITA (1.4%) but not PBO. Rates of hypoglycemia were similar between groups.

Conclusion: Thus BI 10773 resulted in dose-dependent reductions in HbA_{1c}, FPG, body weight and was well tolerated with slightly increased frequency of genital infections but not UTIs vs PBO.

		ΔHbA _{1c} , % vs PBO	ΔHbA _{1c} , % from baseline	ΔFPG, mg/dL	ΔBody Weight, kg
BI 10773	PBO		0.15 (0.08)	4.75 (3.48)	-1.16 (0.31)
	1mg	-0.24 (0.10)*	-0.09 (0.08)*	-1.70 (3.49)	-1.55 (0.31)
	5mg	-0.39 (0.10)***	-0.23 (0.08)***	-15.84 (3.45)****	-2.28 (0.31)**
	10mg	-0.71 (0.10)****	-0.56 (0.08)****	-22.14 (3.49)****	-2.74 (0.31)***
	25mg	-0.70 (0.10)****	-0.55 (0.08)****	-26.83 (3.47)****	-2.56 (0.31)***
	50mg	-0.64 (0.10)****	-0.49 (0.08)****	-27.91 (3.46)****	-2.85 (0.32)****
SITA	PBO		0.13 (0.10)	5.31 (3.75)	-1.16 (0.31)
	100mg	-0.58 (0.12)****	-0.45 (0.10)****	-12.18 (3.73)**	-0.84 (0.31)

All data are mean (SEM). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs PBO

Clinical Trial Registration Number: EudraCT 2008-000641-54

Supported by: Boehringer Ingelheim

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A novel potent and highly selective renal sodium-glucose co-transporter 2 (SGLT2) inhibitor, TS-071, improves glycaemic control and lowers body weight in Japanese patients with type 2 diabetes mellitus

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Background and aims: Renal sodium-glucose co-transporter 2 (SGLT2) inhibition is known as a new approach for the treatment of type 2 diabetes mellitus (T2DM). TS-071 is a novel, orally bioavailable, and highly selective SGLT2 inhibitor. The aim of this study was to investigate the exploratory efficacy and safety in Japanese patients with T2DM.

Materials and methods: In double-blind, placebo (PBO) -controlled, parallel group, exploratory dose-ranging study, subjects (N=236) were randomized to TS-071 0.5, 2.5, 5 mg once daily (QD) or PBO for 12 weeks. The primary endpoint was the change in HbA_{1c} from baseline after 12 weeks of treatment.

Results: Mean baseline characteristics in each group (HbA_{1c} 7.91-8.17 %, fasting plasma glucose (FPG) 152.0-159.9 mg/dL, postprandial plasma glucose at 2-hours after meal-test (PPG) 235.3-254.9 mg/dL, age 55.1-58.3 years, BMI 24.5-25.5 kg/m², body weight 65.4-69.7 kg) were similar across treatment groups. HbA_{1c} decreased significantly and dose-dependently, differences from PBO were -0.43, -0.70 and -0.82 % in 0.5, 2.5 and 5 mg groups respectively. Both FPG and PPG decreased significantly. Differences from PBO in FPG were -14.6, -25.9 and -27.9 mg/dL, and those in PPG were -36.1, -43.0 and -57.3 mg/dL in 0.5, 2.5 and 5 mg groups respectively. Body weight significantly decreased in 2.5 and 5 mg groups compared to PBO. Differences in body weight change compared to PBO were -1.8 kg (about 3 % of total body weight) in both 2.5 and 5 mg groups respectively. There was a trend to modestly systolic blood pressure reductions without relevant change in pulse in 2.5 and 5 mg groups. No major or serious safety concern was observed in all TS-071 groups. No hypoglycaemia (less than 70 mg/dL blood glucose level) was observed. Six pollakiuria or urine output increase were observed in

2.5 and 5 mg groups however all events were mild in severity. There were no clinically meaningful changes in serum creatinine or cystatin C, electrolytes.

Conclusion: Once daily administration of TS-071 demonstrated clinically meaningful reduction in HbA_{1c} and other glycemic parameters. Furthermore TS-071 induced significant body weight reduction, and showed favorable safety profile.

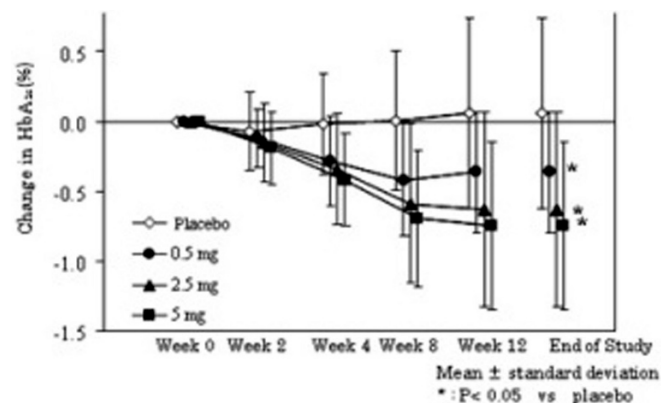


Figure 1. Time-Course of Changes in HbA_{1c}

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Ipragliflozin improved glycaemic control with additional benefits of reductions of body weight and blood pressure in Japanese patients with type 2 diabetes mellitus: BRIGHTEN Study

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Background and aims: Ipragliflozin (ASP1941) is a novel and selective sodium-dependent glucose co-transporter 2 (SGLT2) inhibitor. Here we report the results of a double-Blind Randomized study of Ipragliflozin to show the efficacy as monotherapy in Type 2 diabetes mellitus (T2DM) patients, the BRIGHTEN study; the first report of Japanese phase 3 study with a SGLT2 inhibitor.

Materials and methods: This study consisted of a randomized placebo-controlled parallel-group trial with a 16-week follow-up study in Japanese patients with T2DM. The primary objective of the study was to assess the efficacy (superiority versus placebo) of 50 mg ipragliflozin based on HbA_{1c} changes from baseline. After a 4-week screening period followed by a 2-week placebo run-in period, patients were randomized in strata depending on washout of previous anti-diabetic drugs or drug-naivety (8 weeks prior to screening), and received either ipragliflozin 50 mg (n=62) or placebo (n=67) once-daily for 16 weeks. At the end of treatment, patients were followed up for further 4 weeks.

Results: Mean baseline HbA_{1c} levels (8.32%) were significantly decreased (p<0.001) by 1.23% in the ipragliflozin group, as compared with a placebo group after 16 weeks of treatment. In addition, 43.5% of patients in the ipragliflozin group achieved the HbA_{1c} target goal of <7.4% as compared with 4.5% of patients in the placebo group. Fasting plasma glucose was also significantly decreased in ipragliflozin group, but adiponectin was significantly (p=0.001) increased; and fasting insulin tended to decrease. Mean body weight was significantly (p<0.001) decreased in the ipragliflozin group (-1.47kg as compared with placebo) and there was also reduction of systolic blood pressure (BP) (mean=-3.2 mmHg) and in diastolic BP (mean=-2.5 mmHg) from baseline. The overall incidence of treatment emergent adverse events was comparable between two groups. One mild case of symptomatic hypoglycemia was observed in the ipragliflozin group. One urinary tract infection was observed in the placebo group but none in the ipragliflozin group. Two genital infections were observed in the ipragliflozin group but none in the placebo group. **Conclusion:** The BRIGHTEN study demonstrates that once daily 50 mg of ipragliflozin treatment is safe, well tolerated, and superior to placebo in Japanese T2DM patients, with a significant decrease in HbA_{1c} of 1.23% versus placebo, as well as the additional benefits of weight loss and BP reduction.

Table: Baseline values and adjusted mean change from baseline at Week 16*

	HbA _{1c} [†] (%)	FPG (mg/dL)	Adiponectin (μg/mL)	Fasting insulin (μU/mL)	Body weight (kg)
Mean at baseline	8.32	175	6.29	7.57	67.18
Change from baseline at Week 16 [‡]					
Ipragliflozin adjusted mean ± SE	−0.76 ± 0.11	−39.9 ± 3.5	+0.29 ± 0.11	−1.28 ± 0.51	−2.36 ± 0.23
Placebo adjusted mean ± SE	+0.47 ± 0.10	+5.9 ± 3.4	−0.22 ± 0.11	−0.08 ± 0.50	−0.89 ± 0.22
Adjusted mean difference to placebo (95% CI)	−1.23 (−1.515 to −0.938)	−45.8 (−55.50 to −36.10)	+0.51 (+0.208 to +0.812)	−1.20 (−2.608 to +0.214)	−1.47 (−2.098 to −0.846)
p-Value	<0.001	<0.001	0.001	0.096	<0.001

*Last observation carried forward.

[†]HbA_{1c} levels were measured as defined by the Japan Diabetes Society (JDS) and converted to HbA_{1c} as defined by the National Glycohemoglobin Standardization Program (US and European Union standard) by adding 0.4% to the JDS data.[‡]The results shown are from an analysis of covariance (ANCOVA) model including baseline as covariate, and treatment group and treatment with oral hypoglycemic agents within 8 weeks before screening as fixed effects.

CI = confidence interval; FPG = fasting plasma glucose; SE = standard error.

Clinical Trial Registration Number: NCT01057628

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Single doses of LX4211, a dual inhibitor of SGLT1 and SGLT2, improves parameters of glycaemic control and increases GLP-1 and PYY in patients with type 2 diabetes

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Background and aims: LX4211 is a dual inhibitor of sodium-glucose co-transporters SGLT1/SGLT2, designed to block glucose (G) absorption in the GI tract via SGLT1 inhibition and renal G reabsorption via SGLT2 inhibition. This study compared the PK and PD of a liquid and solid formulation of LX4211 in T2D. PD endpoints included biomarkers hypothesized to reflect (1) inhibition of SGLT1 including total and active GLP-1, PYY; (2) inhibition of SGLT2, 24-hr urinary G; and (3) cumulative effect on glycemic parameters including fasting plasma G (FPG), mean plasma G (MPG), and insulin.

Materials and methods: After a 14-day washout of metformin, patients received single 300 mg oral doses of LX4211 before breakfast as two (2) 150 mg tabs, six (6) 50 mg tabs, or 300 mg solution in a randomized sequence using an open-label, Latin Square crossover design, with a 5-day washout between doses. PK and PD endpoints were assessed on days -1 (baseline [BL] untreated control), 1, 6, and 11.

Results:

	2x150 mg			6x50 mg			300mg liquid		
	BL	Day of Dose	Change from BL	Day of Dose	Change from BL	Day of Dose	Change from BL	Day of Dose	Change from BL
FPG (mg/dL) ^a	161.3	142.6	−18.7 ^c	131.4	−29.9 ^a	129.6	−31.7 ^a		
MPG (mg/dL) ^a	201.9	174.9	−27.0 ^a	171.0	−30.9 ^a	171.5	−30.4 ^a		
Insulin (μU•hr/mL) ^{**}	563.5	480.4	−83.1 ^a	492.9	−70.6 ^a	462.7	−100.8 ^a		
Total GLP-1 (pmol•hr/L) ^{**}	85.3	99.2	13.9 ^c	100.3	15.0 ^a	99.5	14.2 ^a		
Active GLP-1 (pmol•hr/L) ^{**}	42.3	49.5	7.2 ^b	51.1	8.8 ^b	45.3	3.0		
Total PYY (pmol•hr/L) ^{**}	387.8	484.5	96.7 ^c	511.6	123.8 ^a	505.1	117.3 ^a		
24-hr urinary G (g) ^a	17.3	73.1	55.8 ^a	77.5	60.2 ^a	84.8	67.5 ^a		

^aP<0.001^bP<0.05^cP<0.01^a2 hrs post dose^aHrs 2–13 after dosing^{**}Mean AUC for the day

Conclusion: PK and PD were similar between the formulations of LX4211 with favorable changes observed in indices of glycemic control including reduction of FPG, MPG, and insulin. LX4211 produced significant increases in total and active GLP-1, PYY, and urinary G excretion. AEs were infrequent. Single doses of LX4211 markedly increased GLP-1 and PYY and rapidly im-

proved FPG and MPG, consistent with the hypothesis that dual inhibition of SGLT1 and SGLT2 may provide an effective means of treating T2D. Further study of LX4211 as a potential treatment for diabetes is warranted.

Clinical Trial Registration Number: NCT01188863

OP 26 Glycaemia and cardiovascular disease

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Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga diabetes study

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Background and aims: The relationships between the parameters of blood glucose control, cardiovascular events and mortality in type 2 diabetes mellitus are a matter of strong debate. In a previous study, we demonstrated that postprandial blood glucose predicts cardiovascular events in a cohort of type 2 diabetic patients followed-up for 5 years also after adjustment for glycosylated haemoglobin A1C (A1C) and the main cardiovascular risk factors. Aim of the present study was to evaluate whether the predictive power of postprandial blood glucose persists in a long-term follow-up and whether postprandial blood glucose also predicts all-cause mortality.

Materials and methods: 505 consecutive type 2 diabetic patients followed-up at our diabetes clinic (M=53.1%; age: 62.2±9.6 years; known diabetes duration: 9.4±8 years; previous cardiovascular events: 16.8%; 44.6% on diet alone, 43.0% on oral agents, 7.3% on oral agents and insulin, 5.1% on insulin alone) were evaluated at baseline (in 1995) for the main cardiovascular risk factors and for five blood glucose (BG) control parameters (fasting BG, BG 2 hours after breakfast, BG 2 hours after lunch, BG before dinner and A1C); all-cause mortality and the first cardiovascular events occurring during a 14-year follow-up were measured. The statistical analysis has been carried out by Cox analysis with backward method. Non categorical variables were categorized according to the achievement of the therapeutic targets of the American Diabetes Association (ADA) (for BG variables: A1C <7%, preprandial BG ≤130 mg/dl and postprandial BG <180 mg/dl) or evaluated as continuous variables.

Results: We observed 172 cardiovascular events (34.1% of the population) and 147 deaths (29.1% of the population). Categorizing the variables according to the achievement of ADA therapeutic targets, we observed that: a) when the five glycaemic control parameters were considered together, the following resulted predictors: i) for cardiovascular events, BG 2 hours after lunch (HR:1.507, p=0.010) and A1C (HR:1.792, p=0.002); ii) for all-cause mortality, BG 2 hours after lunch (HR:1.885, p=0.000) and A1C (HR:1.907, p=0.002); b) when BG 2 hours after lunch and A1C were considered together with the main cardiovascular risk factors (gender, age, diabetes duration, body mass index, systolic and diastolic blood pressure, smoking status, LDL and HDL-Cholesterol, Triglycerides, Creatinine, Albumin Excretion Rate) the following glycaemic control parameters resulted predictors: i) for CV events BG 2 hours after lunch (HR:1.452, p=0.021) and A1C (HR:1.732, p=0.004); ii) for all-cause mortality, BG 2 hours after lunch (HR:1.846, p=0.001) and A1C (HR:1.896, p=0.004). By evaluating the non categorical parameters as continuous variables, the predictive power of A1C and BG 2 hours after lunch persisted for both CV events and all-cause mortality.

Conclusion: In type 2 diabetic patients, both postprandial blood glucose and A1C -but not fasting blood glucose- predict cardiovascular events and all-cause mortality in a long-term follow-up. Postprandial blood glucose, therefore, should be considered as a useful tool in the cardiovascular risk assessment in type 2 diabetes.

Supported by: Piedmont Region Grants to FC and MT

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The effect of changes in HbA_{1c} during 7 years on aortic stiffness in a population at high diabetes risk: ADDITION DK

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Background and aims: Aortic stiffness is an independent risk factor for cardiovascular disease and is increasingly used as an intermediate end point in clinical trials. Diabetes and hyperglycaemia are associated with higher aortic stiffness, but whether changes in glycaemia affect aortic stiffness remains unknown. The aim of this study was to examine the effect of changes in HbA_{1c} on aortic stiffness in a population at high diabetes risk.

Materials and methods: A Danish population at high risk of diabetes identified by a multi-stage screening programme in general practice had measurements of HbA_{1c} at baseline and at follow-up 7 years later. Assessment of aortic stiffness by carotid-femoral pulse wave velocity (PWV) was performed at the follow-up examination. The effect of changes in HbA_{1c} from baseline to follow-up on PWV was analysed by linear regression analysis adjusted for age, sex, mean blood pressure at follow up, baseline waist circumference, baseline HbA_{1c}, incident diabetes (WHO 1999 definition), and antidiabetic treatment. Participants identified with diabetes based on an OGTT at baseline were not included in the analysis.

Results: A total of 896 participants free of diabetes at baseline (mean age: 60.1 years (SD: 5.9), 56% men) attended the baseline and follow-up examination. Mean follow-up time was 7.1 years (SD: 1.1). Baseline HbA_{1c} was 5.7% (SD: 0.4) and mean change in HbA_{1c} per year was 0.02% point (SD: 0.07, or range: -0.2 to 0.8). During follow-up 188 participants were diagnosed with diabetes, of which 54 participants were treated with antidiabetic medication. A yearly increase in HbA_{1c} of 0.1% point was associated with a 0.24 m/s (95% CI: 0.04;0.45) higher PWV at follow up. The effect of age on PWV was 0.12 m/s (95% CI: 0.09;0.14) per year. Leaving out participants with incident diabetes slightly elevated the effect of change in HbA_{1c} on PWV: 0.36 m/s (95% CI: 0.09;0.62), whereas the effect of age remained unaltered. The effect of change in HbA_{1c} on PWV was independent of baseline HbA_{1c} level.

Conclusion: People without diabetes who experience a faster increase in HbA_{1c} over 7 years have a higher level of aortic stiffness at the end of this period. In this high risk population, the effect of a yearly 0.1% point faster increase in HbA_{1c} on aortic stiffness corresponds to the effect of being 2 years older. This study suggests that not only the current HbA_{1c} level but also the velocity of HbA_{1c} deterioration plays a role in the development of early macrovascular changes in people at high diabetes risk.

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Association of HbA_{1c} with the risk of cardiovascular events in patients with type 2 diabetes: a cohort study of 40,204 patients in primary care

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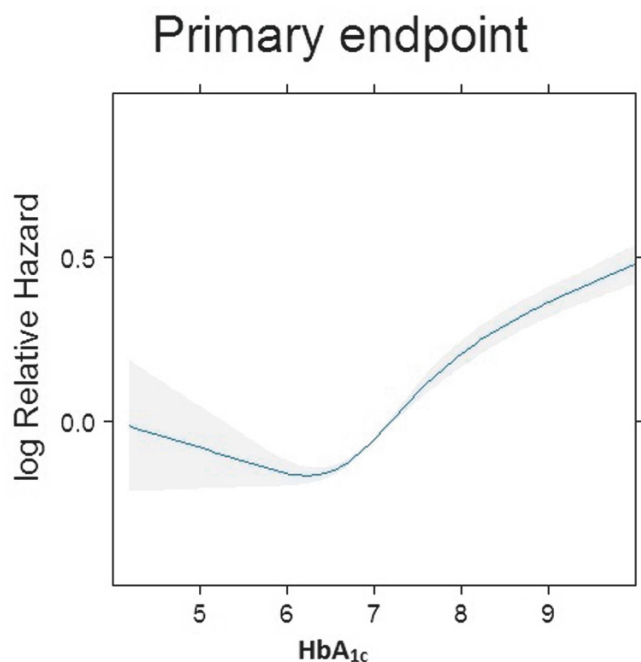
Background and aims: Previous results of both interventional and observational studies in patients with type 2 diabetes have led to conflicting results about the safety of aiming for normal blood glucose concentrations. We explored the association of HbA_{1c} with risk of cardiovascular events (CVD) in patients with type 2 diabetes.

Materials and methods: We identified a cohort of 40,204 patients with type 2 diabetes aged 35 years and older by in 2010 extracting data from electronic patient records for all patients who had a diagnosis of type 2-diabetes or had glucose lowering agents prescribed between 1999 and 2008 in 76 Swedish primary care centers. In addition, the data was linked to the Statistics Sweden, National Cause of Death- and National Hospital Discharge Register. Risk of a primary composite endpoint of major cardiovascular events (acute

myocardial infarction, heart failure, stroke, or cardiovascular death) was estimated by Cox regression with HbA_{1c} as annual updated means as exposure. In the model, age and systolic blood pressure were entered as annual updated means, gender and previous CVD level as fixed covariates.

Results: During follow-up, 10,018 (24.9 %) patients experienced a major cardiovascular event. The relation of HbA_{1c} to risk of major cardiovascular events was J shaped (figure), with the lowest risk observed at an HbA_{1c} level of 6.3 % (45 mmol/mol). When further adjusted for index year and three categories of educational level the results were virtually unchanged.

Conclusion: In this observational retrospective cohort study we were able to confirm some previous results reporting that low and high mean HbA_{1c} values were associated with increased major cardiovascular events and cardiovascular mortality. The results lend support to the hypothesis that future diabetes guidelines might include a minimum HbA_{1c} value for patients subjected to treatment with glucose lowering agents with the potential of inducing hypoglycemia.



Clinical Trial Registration Number: NCT01121315
Supported by: AstraZeneca

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Glucose lowering therapy and long-term mortality in patients with type 2 diabetes undergoing coronary angiography

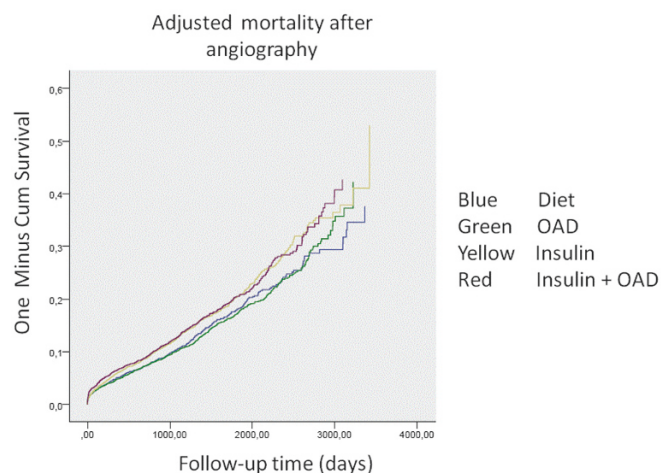
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Background and aim: Patients with type 2 diabetes mellitus (DM) undergoing Percutaneous Coronary Intervention (PCI) are at increased mortality risk compared to those without and insulin treatment has been discussed to be associated with adverse prognosis after PCI. The aim was to investigate the association between glucose lowering treatment and long-term mortality in patients with type 2 DM undergoing coronary angiography adjusting for diabetes variables indicating a more severe diabetes state as well as for cardiovascular risk factors.

Materials and methods: By merging the Swedish coronary angiography and angioplasty registry (SCAAR) and the National Diabetes registry (NDR) 14080 patients were identified in both registries between the years 2001–2009. Long-term mortality until end of 2009 was analysed by glucose lowering treatment adjusting for baseline cardiovascular-, diabetes- and angiographic characteristics.

Results: Patients with insulin only and insulin in combination with oral glucose lowering drugs at baseline had more severe coronary artery disease and higher rate of microvascular diabetes complications. Multivariate analysis, including diabetes variables as HbA_{1c} and duration, identified treatment with insulin (OR 1.19 95% CI 1.03–1.37, $P < 0.01$) and insulin in combination with oral anti diabetes drugs (OR 1.22 95% CI 1.06–1.40, $P < 0.005$) as predictors for long-term mortality. **Conclusion:** Insulin treatment alone or in combination with oral anti diabetic drugs in patients with type 2 DM admitted for angiography is associated with higher risk for mortality and such patients should be regarded as a high risk population. The role of insulin per se in this context needs to be better explored.



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Population attributable risk (PAR) of macrovascular events associated with HbA_{1c} , blood pressure or weight in patients with type 2 diabetes mellitus: evidence from a Dutch cohort

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Background and aims: To determine the PAR of macrovascular events associated with HbA_{1c} , blood pressure, or weight in patients with type 2 diabetes mellitus (T2DM).

Materials and methods: A population-based, patient centric data tracking system in the Netherlands, the PHARMO database, was used which includes complete information on drug dispensing, hospital morbidity, clinical laboratory and primary care diabetes monitoring data for approximately 11,000 patients. From this dataset, all T2DM patients monitored in 2000–2008 were included in a cross-sectional cohort if cardiovascular risk factors HbA_{1c} , SBP and BMI were assessed during a one year baseline period after at least 6 months of antidiabetic treatment. Patients without baseline macrovascular events were followed until monitoring ended. Cox regression modeling of the composite outcome of macrovascular events was used to estimate the expected number of events after 2 and 5 years, either with unchanged risk factors (base-case) or with reductions in risk factors. PAR is the percent incidence of an outcome in the population that would be eliminated if a risk factor was reduced, compared with its actual exposure pattern. PAR was calculated as the number of averted events divided by the number of expected events in the base-case analysis.

Results: Mean age of 5841 included patients was 66 years (55% male), 45% had HbA_{1c} levels $\geq 7\%$, 66% had a SBP ≥ 140 mmHg and 85% had a BMI ≥ 25 kg/m². The base case expected number of macrovascular events at 2 years was 333, and 284 after reduction to target of all 3 risk factors. The overall PAR of HbA_{1c} , SBP and BMI for the composite outcome of macrovascular events was 15%, ranging from 6% among those with only one elevated risk factor to 23% among those with all three elevated risk factors. At 5 years, the base case expected number of macrovascular events was 796, and 687 after reduction to target of all 3 risk factors the PAR was 14% overall, ranging from 5% among those with only one elevated risk factor to 21% among those with all three elevated risk factors. The PAR of reducing HbA_{1c} to target (7%) or by 0.5

percent points ranged from 2–11% at 2 years, and 2–10% at 5 years. The PAR of reducing SBP to target (135 mmHg) or by 10 mmHg ranged from 3–13% at 2 years, and 3–12% at 5 years. There was no meaningful effect of reduction in BMI alone. However, meaningful effects of HbA_{1c} or SBP reduction were observed in patients with high BMI.

Conclusion: Reducing elevated HbA_{1c} and blood pressure levels was associated with improvements in cardiovascular risk. Even modest reductions in risk factors lead to significant reductions in macrovascular events in patients with T2DM.

Supported by: AZ

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Risk of cardiovascular disease associated with sulphonylurea or metformin use in older patients with type 2 diabetes mellitus

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Background and aims: Sulphonylurea therapies have been associated with the potential for increased risk of cardiovascular disease (CVD), but remain a commonly used antihyperglycaemic medication particularly in the elderly. This retrospective cohort study examined the potential association between initial monotherapy with sulphonylurea or metformin and subsequent CVD in older patients with type 2 diabetes mellitus (T2DM).

Materials and methods: A cohort of patients who were ≥65 years old with T2DM and received their first prescription of sulphonylurea or metformin monotherapy between 2003 and 2007 (index period) and remained on it for at least 90 days were selected from the GE Centricity electronic medical record database. Patients had to have no prescriptions for antihyperglycaemic agents and no CVD events (ischaemic heart disease [IHD], myocardial infarction, stroke, transient ischaemic attack, and peripheral arterial disease) within the 1-year period prior to the index date (baseline) and also had at least 2 years of follow-up after the first prescription. Logistic regression evaluated the likelihood of having a CVD event. Cox regression estimated time to first CVD event. Differences in baseline characteristics (demographics, clinical and laboratory measures, and comorbidities) were controlled for using propensity score matching (PSM).

Results: Overall, 8,502 patients were included with 4,251/group. Mean age was 75 years and 49% were males. While controlling for differences in baseline characteristics using PSM, patients who initiated with a sulphonylurea had a significantly ($p<0.001$) higher incidence of CVD events (12.4% vs. 10.4%) compared to those initiated with metformin after 2 years of follow-up. The difference was mainly driven by the increased incidence of IHD with sulphonylurea (7.2%) compared to metformin (5.5%) ($p=0.002$). The likelihood of having a CVD event was higher in patients initiated with sulphonylurea than with metformin (odds ratio [95% CI] = 1.23 [1.08, 1.41]; $p=0.002$). Sulphonylurea use was associated with shorter time to first CVD event compared to metformin (hazard ratio [95% CI] = 1.15 [1.03, 1.28]; $p=0.004$). Sensitivity analyses with 1 or 3 years of follow-up yielded similar results.

Conclusion: In a cohort of older patients with T2DM initiating antihyperglycaemic therapy, the likelihood of experiencing a CVD event was higher and these events occurred sooner in patients who started with sulphonylurea monotherapy than those who started with metformin.

Supported by: MSD, Whitehouse Station, NJ

OP 27 Diagnosis and natural history of type 1 diabetes

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EURODIAB childhood type 1 diabetes incidence registers - results from the first 20 years

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Background and aims: In 1989 a number of registries in Europe began recording new cases of type 1 diabetes diagnosed in children aged under 15 years using a common protocol as part of the EURODIAB sub-area A project. Trends in incidence rate during the first 20 years of registration are described covering the period 1989–2008.

Materials and methods: All registries operate in geographically defined regions and are based on a clinical diagnosis. When possible, registries assess their completeness by capture-recapture methodology using a primary and a secondary source of ascertainment and noting the numbers of cases identified by the primary source only, by the secondary source only and by both the primary and the secondary source.

Results: Other registries have joined the group since 1989, and 21 registries in 15 countries continue to submit registration data. In the first five years (1989–93) incidence rates varied from 3.2 per 100,000 in the Former Yugoslav Republic of Macedonia to 25.8 per 100,000 in the Stockholm area of Sweden. In the last five years (2004–2008) these two registries again had the lowest and highest incidence, but rates had increased to 5.8 per 100,000 and 36.6 per 100,000, respectively. During the 20 year period all but one of the 21 registries showed statistically significant rates of increase (median rate of increase 4% per annum), and similar figures were obtained when this median rate of increase was estimated for the first half of the period (1989–1998) and for the second half (1999–2008). However, rates of increase differed significantly between the first half and the second half of the period for eight of the 17 registries with adequate coverage of both periods; four showed significantly higher rates of increase in the first half and four significantly higher rates in the second half.

Conclusion: The childhood type 1 diabetes incidence rate continues to rise across Europe by approximately 4% per annum, but the increase in a registry is not necessarily uniform with periods of less rapid and more rapid increase in incidence occurring in some registries. This pattern of change suggests that important risk exposures differ over time in different European countries. Further time trend analysis and comparison of the patterns in defined regions is warranted.

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Identification and assessment of glycan profiles as a biomarker for maturity onset diabetes of the young (MODY) due to hepatocyte nuclear factor 1 alpha (HNF1A) mutations

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Background and aims: A molecular diagnosis of monogenic diabetes confers important clinical benefits; however the majority of HNF1A-MODY patients remain misdiagnosed. Biomarkers could help identify subjects for genetic testing. A recent genome-wide association study identified HNF1A as the master regulator of plasma protein fucosylation. Enhanced HNF1A transcriptional activity is associated with an increased proportion of plasma glycans containing antennary fucose. We hypothesised that inactivating HNF1A

MODY mutations would be characterised by reduced antennary fucosylation of plasma proteins. We aimed to assess the value of protein fucosylation as a biomarker for HNF1A-MODY.

Materials and methods: Glycan plasma or serum profiles were analysed by HPLC in a pilot study of 33 HNF1A-MODY and 41 type 2 diabetes ([T2DM], diagnosed <45 years) subjects. Glycan peaks providing optimum discrimination between diabetes subtypes were validated in an independent dataset: HNF1A-MODY (n=199), GCK-MODY (n=127), HNF4A-MODY (n=44), type 1 diabetes (T1DM, n=98), T2DM (n=173) and non-diabetic controls (n=98). Receiver operating characteristic (ROC) curve analysis was used to evaluate the discriminative power of antennary fucosylation with respect to diabetes aetiology. Subjects with T1D or T2D with glycan profiles overlapping with HNF1A-MODY (n=37) underwent HNF1A sequencing.

Results: In the pilot study the ratio of glycan peak GP13 (contains glycans mostly without antennary fucose) to glycan peak DG9 (contains mainly antennary fucosylated glycans) levels were significantly higher in HNF1A-MODY compared with T2DM subjects; 4.9 vs. 1.3, $p < 5 \times 10^{-8}$. In the validation group the GP13:DG9 levels were substantially higher than all other groups ($p < 5 \times 10^{-8}$). Median (IQR) GP13:DG9 levels were: HNF1A-MODY 4.90 (2.88–7.14); GCK-MODY 1.43 (1.00–2.33); HNF4A-MODY 2.08 (1.04–4.11); T2DM 1.19 (0.75–2.05); T1DM 1.07 (0.65–1.74) and non-diabetic controls 1.24 (0.79–1.63). C-statistic was 0.90 for HNF1A-MODY vs. T2DM and 0.93 for HNF1A-MODY vs. T1DM indicating that glycan profiles confer high discriminative accuracy between diabetes subtypes. HNF1A sequencing identified 2 diabetic subjects (1 T1D & 1 T2D) with previously reported mutations consistent with a diagnosis of HNF1A-MODY.

Conclusion: We provide the first evidence that glycan profiles provide discrimination between HNF1A-MODY and other diabetes aetiologies and can identify subjects with unrecognised HNF1A-MODY. Glycan profiles offer a promising biomarker for HNF1A-MODY, which could be developed into a robust screening tool.

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Parietal cell autoantibodies in type 1 diabetes based on molecularly-defined antigen epitopes

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Background and aims: Around 10–20% of type 1 diabetic patients are reported to express circulating gastric parietal cell autoantibodies, a classical marker for atrophic body gastritis (ABG) marker found in 2% of the general population which may lead to manifest clinical manifestation of pernicious anemia (PA) 0.15–1%. Current PCA assays typically rely on immunohistochemical detection to human stomach sections or ELISA based on binding of autoantibodies to H+/K+ ATPase (ATP4) biochemically purified from porcine stomach microsomes. A key question is whether PCA antibodies in T1D recognize the same or a homologous antigen in islets. To address this issue we developed assays for antibodies directed at recombinantly expressed alpha and beta subunits to human (ATP4) and their subdomains predicted from 3D modeling of ATP4A on the closely-related Na+/K+ ATPase. Further refinement and optimization of the assay to defined epitopes was performed by truncation analysis and site-directed mutagenesis.

Materials and methods: pCDNA 3 constructs were 35S-methionine labeled in vitro transcription / translation and incubated with sera derived from 463 newly diagnosed T1D individuals (9.8±4.4yr old), 150 patients with diabetes of >20 yr duration (30.4±8.7yr old) and 212 controls (median 6.3±13.2yr; range 0.7–51yr) and 60 patients with bioscopically confirmed ABG (55±14.8yr old). Immune complexes were captured on Protein A coated agarose bead radioactivity determined by scintillation counting in a96 well format.

Results: A major antigenic determinant of T1D autoantibodies to ATP4A was mapped to a 30aa epitope within 230aa domain within an intracellular loop of the molecule. 26% recent onset patients tested positive vs. 6% of control subjects. Significant reactivity at a low titer was observed in 5% T1D subjects with an equivalent domain of ATP12A, the most-closely related (78% identity) H+/K+ ATPase found in skin and other tissues but not to Human Na/K ATPase (ATP1A) above a 99% cut off (191 samples) and more distantly related Ca ATPases. In contrast to the gold standard T1D autoantibodies (GAD65, insulin, IA-2, and ZnT8) whose titers generally decrease with age and duration of T1D, ATP4A prevalence increased with age of onset of T1D from an average of 8% prevalence at 5 yr age to >30% at 60 yr. Immunoreactivity to ATP4A, unlike that of T1D antigens, displays a significant gender

bias in newly diagnosed T1D individuals and longstanding disease (60/40 ratio). Comparison of the ATP4A RIA with that of standard PCA commercial ELISA using sera from 60 individuals with ABG showed higher sensitivity (95% vs 80% respectively at 95% specificity) but otherwise excellent concordance in titer. Quantitative PCR and sequence analysis of human islet cDNA failed to detect ATP4A.

Conclusion: We demonstrate that assays developed to molecularly defined epitope of ATP4A that is detected in 8–30% of T1D patients is equivalent to the major epitope found in ABG patients. The antigen is not expressed in islets to a significant extent nor does the assay detect antibodies to structurally-related homologous ATPases which are found in islets. The association of PCA with type 1 diabetes is likely to be attributable to a more generalized polyendocrine autoimmune response arising from a defect in central immune tolerance.

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Analyses of the rate of decline in stimulated C-peptide 12 months after diagnosis in children with newly diagnosed type 1 diabetes: results from 4 European cohorts during a period of 20 years

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Background and aims: The aim was to characterize the natural history of the decline in stimulated C-peptide (SCP) the first 12 months after diagnosis in children with new onset type 1 diabetes (T1D) in four independent cohorts collected over a time interval of 20 years and construction of a historic control group with the aim to reduce the required placebo group in paediatric intervention studies.

Materials and methods: The Wallensteen Swedish cohort, yr 1986–1988: 39 children; the Örtqvist Swedish cohort, yr 1995–1998: 29 children; the International Hvidoere European cohort, yr 1999–2000: 262 and the Danish cohort, yr 2005–2006: 130 children. All patients went through a 90-minutes liquid mixed meal test 1, 3 (not the Hvidoere cohort), 6 and 12 months after onset to characterize the residual beta-cell function. In all children ≥ 5 years the linearity of the slope of decline in SCP was analyzed from 3–12 months on a logarithmic scale. Linear mixed-effect models (random coefficient regression) were used to determine cohort differences.

Results: Maximum values of SCP were reached three months after diagnosis. Thereafter there was a linear decline in SCP for all age groups ≥ 5 years in all cohorts. The mean slope for the rate of decline in SCP was 7.5 ± 1.7 %/month for the Wallensteen cohort, 5.7 ± 1.9 %/month for the Örtqvist cohort, 7.2 ± 0.8 %/month for the Hvidoere cohort and 9.3 ± 1.0 %/month for the Danish cohort. The differences in slope between the cohorts was not statistically significant ($p=0.18$). The combined disappearance rate for SCP for the four cohorts was 7.8 ± 0.6 %/month. The combined standard deviation for the slope for one individual: 0.0997%/months measured 3, 6 and 12 months after diagnosis can be used in the construction of a historic control group based on the present data. With a target difference for the decline in stimulated C-peptide at 4 %/month, corresponding to 50 % reduction in slope, we want 80 % power to demonstrate a difference in fall in SCP at a 5 % level. Using the combined cohorts described above as a historical placebo group we only need 58 patients in the intervention group.

Conclusion: Residual beta cell function as assessed by SCP decline appears to have remained unchanged over time in Europe during the last 20 years despite more intensive insulin treatment regimens and better glycaemic control have been introduced during this period of time. With the construction of a historic placebo control group the number required in the intervention group can be reduced to 58 patients compared to 99 patients per group (198 in total) in a standard two-group comparison. Besides it is possible to randomize relatively fewer patients to the placebo group for confirmation within a study if desired.

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Time dependent C-peptide decline in 4411 patients with recent onset type 1 diabetes followed for up to 10 years: a meta-analysis from 8 European centres

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Background and aims: C-peptide is a valuable indicator of residual beta cell function in patients with type 1 diabetes (T1D). Critical factors associated with decline of C-peptide after diagnosis include age at onset, autoantibody and HLA status, severity of initial metabolic derangement, insulin use and quality of glucose control. Whilst recent studies suggest that significant beta cell function may be preserved for several years after diagnosis, C-peptide data must be corrected for these variables and robust data is lacking. To this aim, we collected retrospectively data on C-peptide from T1D patients from 8 different European centres followed-up from diagnosis and up to 10 years.

Materials and methods: We performed a meta-analysis of 4411 T1D patients in total (57.2% males; mean age at onset: 18.5 yrs. \pm 11.7 SD, age range 1–60). Records were extracted for age at diagnosis, fasting and stimulated C-peptide, Body Mass Index, HbA_{1c} and insulin requirement at diagnosis, and from 1 to 10 years follow-up.

Results: Data on C-peptide (nmol/l) at diagnosis were available from 3648 patients who were subdivided according to age at diagnosis in three groups: Group A n=1356 (0–11 yrs.); Group B n=647 (12–18 yrs.); Group C n=1645 (>18 yrs). There was a significant trend ($P<0.001$) age-dependent increase in fasting C-peptide at diagnosis (0.20 \pm 0.20 in Group A, 0.28 \pm 0.26 in Group B, and 0.30 \pm 0.35 in Group C), not observed for stimulated C-peptide (0.46 \pm 0.43 in Group A, 0.47 \pm 0.32 in Group B and 0.44 \pm 0.38 in Group C). In Group A the percentage decline of baseline C-peptide at 1, 2, 5 and 10 years was 25, 70, 75, 90 percent, respectively; in Group B, 76, 60, 67 and 85 percent, respectively; in Group C, 23, 43, 75 and 77 percent, respectively (p for trend <0.001).

Conclusions: This study (the largest of this kind) reveals an inverse correlation between age at T1D onset and basal C-peptide at diagnosis with a more rapid decline in beta-cell function in the very young patients. The data will be useful for the calculation of treatment effects and sample sizes in trials aiming to preserve insulin secretion with C-peptide as end-point.

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Examination of GAD65 in human serum as a possible marker of ongoing beta cell destruction in autoimmune diabetes

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Background and aims: Autoantibodies (AABs) against beta cell antigens are established serological markers of autoimmune-mediated diabetes, and are detectable at clinical onset but also years in advance. Most frequently are AABs against the 65 kD isoform of glutamate decarboxylase GAD65 (GADA) in both patients with type 1 diabetes (T1D) and latent autoimmune diabetes in adults (LADA), beside AABs against islet cell antigen-2 (IA-2A), insulin (IAA) and zinc transporter 8. GADA are probably secondarily generated after release of GAD65 from injured beta cells. Recently, the detection of GAD65 in serum has been proposed as a non-degradable biomarker for early diagnosis of T1D, and an average concentration of 58 ng/ml (range 0.10 to 514.7 ng/ml) in 64 randomly selected samples from a blood bank was reported. However, previously reported results from own work and another study demonstrated that GAD65 is rapidly degraded in vitro and in vivo. The aim of the present study was the quantification of GAD65 in serum samples from different defined proband groups to evaluate its diagnostic relevance in addition to the established AABs.

Materials and methods: Sera from 103 patients with newly diagnosed T1D (median age 13 years, interquartile range (IQR) 8–20 years) and positivity for GADA (n=82) and/or IA-2A (n=59) and/or IAA (n=38), 93 LADA patients

(median age 59 years, IQR 48–66 years; median latency to insulin treatment 5 years, IQR 2–9 years) and positivity for GADA (n=85) and/or IA-2A (n=20) and 100 healthy blood donors (all negative for GADA and IA-2A) were analyzed. Additionally, sera from 151 schoolchildren positive for GADA (n=86) and/or IA-2A (n=49) and/or IAA (n=46) and 100 AAB negative schoolchildren (median age 11 years, IQR 9–13 years) without first-degree diabetes heredity were included. GAD65 was quantified by a sandwich ELISA based on two monoclonal antibodies with different epitope specificities and a detection limit of 30 pg/ml. Statistical analysis was performed with the non-parametric Mann-Whitney test.

Results: The median GAD65 concentration was significantly higher in T1D patients compared to healthy blood donors (0.949 ng/ml; IQR 0–1.76 ng/ml vs 0.573 ng/ml; IQR 0.32–0.81 ng/ml; $p=0.044$), while levels of LADA patients (0.758 ng/ml; IQR: 0.24–1.25 ng/ml) did not differ from blood donors. Furthermore, the median GAD65 concentration in sera from AAB positive schoolchildren was significantly higher than in sera from AAB negative schoolchildren (0.684 ng/ml; IQR 0.31–1.45 ng/ml vs 0.425 ng/ml; IQR 0.11–0.98 ng/ml; $p=0.0096$). Highest individual values were 5.8 ng/ml (T1D), 16.6 ng/ml (LADA), 4.9 ng/ml (blood donors) and 5.8 ng/ml respectively 2.8 ng/ml (AAB positive/ negative schoolchildren).

Conclusion: The results demonstrate that GAD65 can be detected in sera from patients with newly diagnosed T1D or LADA, from AAB negative and AAB positive schoolchildren and from healthy blood donors. However, the measured concentrations are about 100fold lower than previously published data and differ only slightly between the examined groups. Taken the reported degradation kinetics of GAD65 into consideration, the detection of GAD65 is therefore, at most, only of minor diagnostic relevance in autoimmune diabetes.

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Genome-wide associations between gene expression in peripheral blood and fasting and 2-hour glucose levels in the population-based KORA Survey F4

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Background and aims: Gene expression profiles represent quantitative traits that are inherited, but also determined by environmental influences. However, it is not known to what extent the human transcriptome is influenced by changes of glucose metabolism. As primary glucose-metabolising tissues are not easily accessible in epidemiological studies, we used peripheral blood to assess if gene expression levels were associated with fasting and 2-hour glucose levels after an oral glucose tolerance test.

Materials and methods: Whole-blood samples in PAXgene tubes were obtained between 8 and 11 a.m. from fasting study participants of the population-based KORA Survey F4 (Augsburg/Germany). Whole-genome gene expression analysis was performed using the Illumina HumanHT-12v3 Expression BeadChip which measures the relative abundance of >32,000 transcripts covering the vast majority of the protein-coding genes annotated in the RefSeq database. Associations between expression levels of RefSeq annotated genes and glucose (fasting and 2-hour) were quantified by linear regression models using normalised gene expression levels as dependent variables. Correction for multiple testing was performed using a multiple testing procedure based on Storey critical values which controls the false discovery rate (FDR) at a level of 0.05.

Results: This study is based on expression data from 815 individuals (48.2% men) between 62 and 81 years without previously known diabetes (63.2% normal glucose tolerance, 5.9% impaired fasting glucose, 23.7% impaired glucose tolerance, 7.2% newly diagnosed type 2 diabetes) with a body mass index (BMI, mean±SD) of 28.5±4.3 kg/m². In age- and sex-adjusted analyses, 343 and 1595 transcripts were associated with fasting and 2-hour glucose at an FDR of less than 0.05. After additional adjustment for BMI, these numbers were reduced to 5 and 688, respectively. For fasting and 2-hour glucose, 3 (60.0%) and 444 (64.5%), respectively, of these transcripts were positively associated with glucose levels. The transcripts showing the most pronounced associations with 2-hour glucose represent several biological pathway that include among others insulin resistance, oxidative phosphorylation and cell proliferation.

Conclusion: Gene expression levels in peripheral blood from fasting individuals are more strongly associated with 2-hour than fasting glucose levels. These results will be available for pathway analyses in order to identify novel pathophysiological mechanisms that contribute to type 2 diabetes, and prospective studies will have to assess whether these transcripts improve the prediction of incident type 2 diabetes.

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The association and causal inference between circulating 25-hydroxy vitamin D concentration and the risk of type 2 diabetes

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Background and aims: Epidemiological evidence suggests that circulating 25-hydroxy vitamin D [25(OH)D] is inversely associated with risk of type 2 diabetes (T2D). No meta-analysis of prospective evidence exists and the caus-

al nature of the association is unknown. Our aim was to conduct a systematic review and meta-analysis of prospective studies and examine causality.

Materials and methods: A systematic review identified all prospective studies on 25(OH)D and incident T2D published in MEDLINE or EMBASE until 20 January 2011. We performed a random effects meta-analysis combining available evidence with new results from EPIC-Norfolk. We genotyped four single nucleotide polymorphisms known to be robustly associated with 25(OH)D, and computed a genetic score. In Mendelian randomisation analyses we compared the odds ratio observed for the association between genetic score and T2D (5,791 cases; 7,786 controls) to that expected based on the mean difference in 25(OH)D by genetic score (n=628, Ely Study), and the summary estimate from our 25(OH)D-T2D meta-analysis.

Results: The combined relative risk of T2D comparing the highest with lowest quartile of 25(OH)D was 0.53 (95% CI 0.45, 0.61), with little heterogeneity ($I^2=0\%$, $p=0.91$) between 7 studies included in the meta-analysis (2,654 cases, 44,236 non-case participants). The mean difference in 25(OH)D was -5.81 (95% CI -7.82, -3.79) nmol/l per tertile of genetic score, corresponding to an expected OR for T2D of 1.05 (1.04, 1.07), compared with an observed OR of 1.00 (0.95, 1.05).

Conclusion: Higher 25(OH)D is associated with a reduced risk of T2D, but the absence of a lower risk of T2D in participants with genetically raised 25(OH)D levels raises caution about the causal nature of the 25(OH)D-T2D association. However, genetic approaches alone may be insufficient to resolve the issue of causality. Given the potential public health importance, randomised trials of vitamin D supplementation are warranted to resolve the issue of causality and the potential for prevention.

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Rs10906115 in CDC123/CAMK1D locus and rs1359790 near SPRY2 are associated with susceptibility to type 2 diabetes in a Japanese population

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Background and aims: Recently, new genetic risk variants for type 2 diabetes (T2D), rs10906115 in CDC123/CAMK1D and rs1359790 near SPRY2, have been identified by a genome-wide association study (GWAS) in Chinese populations. To know the role of these variants in conferring susceptibility to T2D in the Japanese, we determined the association of the 2 single nucleotide polymorphisms (SNPs) with T2D in 11,839 Japanese individuals.

Materials and methods: We genotyped these SNPs using 8,719 T2D cases and 3,120 controls enrolled in several medical institutes in Japan. The genotype of each SNP was determined by multiplex PCR-invader assay. To test the additive model of each SNP, genotype distribution differences between the case and control groups were analyzed using a logistic regression analysis with or without adjusting age, sex and log-transformed (ln)BMI. The association of rs10906115 was further evaluated in a conditional analysis using rs12779790, which had been reported by a previous European GWAS of T2D, in the same logistic model for 2,798 cases and 2,403 controls. We also performed quantitative traits analyses for fasting plasma glucose (FPG), homeostasis model assessment (HOMA)- β and HOMA-IR in 1,392 non-diabetic Japanese individuals by multiple linear regression analysis with or without adjusting age, sex and lnBMI.

Results: The observed T2D risk alleles, rs10906115-A and rs1359790-G, were in accord with those previously reported in other East Asian populations, and both of variants were significantly associated with susceptibility to T2D in the present Japanese population (rs10906115; OR = 1.14, 95% CI = 1.08 - 1.21, $p = 9.38 \times 10^{-6}$, rs1359790; OR = 1.13, 95% CI = 1.06 - 1.21, $p = 3.04 \times 10^{-4}$). Adjustment of age, sex and lnBMI did not have significant effect on the association of these SNPs with the disease. The result of conditional analysis revealed

that the association of rs10906115 with T2D ($p=0.001$ before conditioning on rs12779790), remained significant even after conditioning on rs12779790 ($p=0.0051$). We could not observe significant associations of the 2 SNPs with any of BMI, FPG, HOMA- β or HOMA-IR ($p>0.05$).

Conclusion: Rs10906115-A and rs1359790-G had significant association with T2D in a Japanese population. Therefore, these two variants are considered as common susceptibility variants for T2D among East Asian populations.

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Genome-wide joint meta-analysis of SNP by BMI interaction on fasting insulin: a MAGIC study

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Background and aims: Type 2 diabetes (T2D) is a complex disease resulting from both genetic and lifestyle/behavioural factors. A large number of genetic regions associated with T2D or related traits have recently been reported. Most of these appear to be associated with β -cell function rather than insulin resistance (IR). For example, in recent meta-analyses, 16 loci were significantly associated with fasting glucose (FG), but only two were significantly associated with fasting insulin (FI). As body mass index (BMI) is a key risk factor for both IR and T2D, heterogeneity introduced by BMI may obscure genetic IR signals. We aimed to identify novel genetic IR signals by accounting for both BMI and gene by BMI interactions in a genome-wide association study of FI.

Materials and methods: We conducted study-level single nucleotide polymorphism (SNP) by BMI (SNP×BMI) interaction regression models on ln(FI) and meta-analysed SNP and SNP×BMI associations across 29 cohorts comprising up to 51,750 individuals without T2D, using the newly developed joint meta-analysis method. Thirty-one SNPs reaching a threshold of $P<10^{-5}$ were taken forward to replication in 18 additional cohorts comprising up to 27,518 individuals. For these SNPs, we also investigated SNP associations with ln(FI), stratified by level of BMI (less than vs greater than or equal to 28 kg/m²).

Results: We identified 6 genomic regions newly associated with FI at $P<5\times 10^{-8}$ in combined discovery and replication analyses. A SNP near GRB14 was the most significant signal ($P=1.3\times 10^{-19}$), and showed a larger effect size in heavier ($\beta=0.038$, $P=1.6\times 10^{-8}$) compared to lighter individuals ($\beta=0.018$, $P=3.0\times 10^{-5}$) ($P_{\text{interaction}}=0.04$). In contrast, a SNP near IRS1 was associated with FI ($P=4.4\times 10^{-14}$), but showed similar effect sizes in both BMI strata (lighter $\beta=-0.018$, $P=3.8\times 10^{-10}$; heavier $\beta=-0.021$, $P=1.7\times 10^{-5}$). Additional SNPs near PPP1R3B, PDGFC, PEPD, and LYPLAL1 also showed genome-wide significant associations with FI. All newly identified FI-raising alleles were also found to be associated with both lower HDL ($P<0.001$) and higher triglyceride levels ($P<0.02$): a combination which is a hall-mark of IR.

Conclusion: We identified novel genetic signals for IR by accounting for heterogeneity in BMI. These findings demonstrate the importance of accounting for other key risk factors when studying the genetic determinants of complex and multifactorial traits. Results for GRB14 also suggest that variation in BMI may modify genetic associations with IR.

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High fibres intake associates with elevated risk increase of incident type 2 diabetes by TCF7L2 risk variant: evidence for putative mechanism by apoptosis

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Background and aims: TCF7L2 rs7903146 is the strongest and most widely replicated T2D susceptibility locus and has also been shown to associate with increased fasting plasma glucose and HbA_{1c} levels. As at least part of this risk increase has been indicated to be mediated by attenuated insulin response,

possibly due to diminished incretin effect, we investigated if dietary intakes of carbohydrates, fats, whole grains or fibers modify the increased risk of T2D by this variant. We then studied if butyrate, a fermentation product of dietary fibers, may affect apoptosis of β -cells, as this has been reported earlier to occur in colon cancer cells via activation of TCF4 encoded by TCF7L2.

Materials and Methods: Altogether 24,799 non-diabetic individuals (45-74 years) from the Malmö Diet and Cancer cohort with diet data based on a 7-day food diary, a 168-items diet questionnaire and a 1-h diet-history interview were followed-up for 12 years and 1,649 incident T2D cases were recorded. Risk of T2D in strata of diet tertiles and quintiles was analyzed by logistic regression adjusting for potential confounders. Among 5,251 non-diabetic individuals we analyzed association between rs7903146 and fasting plasma glucose and HbA_{1c} in diet tertiles adjusting for potential confounders. Interactions were assessed by adding diet tertiles, genotypes and their multiplicative term to the analysis. INS-1 832/13 cells were cultured for 48 hours in different butyrate concentrations. Cell death detection ELISA kit was used to measure apoptosis level at 100 μ M, 500 μ M, 1mM, 5mM, and 10mM of butyrate. Insulin secretion was measured by a RIA kit at 1mM butyrate concentration after 1 hour stimulation by 2.8 mM and 16.7 mM glucose.

Results: TCF7L2 T-allele was associated with a 44% (95% CI: 33-56%) increased risk of T2D ($p=4.6\times 10^{-19}$) and each additional T-allele associated with 0.070 mmol/L higher fasting plasma glucose ($P=0.001$) and 0.026% higher HbA_{1c} ($P=0.019$). No significant interactions were found between rs7903146 and intake level of carbohydrates, whole grains or fats on T2D risk. The risk of T2D by rs7903146 increased significantly by increasing fibers intake [OR: 1.27, 1.50, and 1.67 for low, medium and high tertiles ($P_{\text{interaction}}=0.05$) and OR: 1.19, 1.42, 1.52, 1.58, and 1.71 from lowest to highest quintiles ($P_{\text{interaction}}=0.006$)]. The associated effect size per each T-allele on HbA_{1c} increased with increasing fibers and whole grains intakes ($P_{\text{interaction}}=0.088$ and 0.044, respectively). Apoptosis level of INS-1 832/13 increased by rising butyrate concentrations ($p<0.0001$), with a significant increase at 1mM ($p=0.004$). Insulin secretion of INS-1 832/13 was 6-fold ($P=3\times 10^{-6}$) and 3-fold ($P=5\times 10^{-4}$) higher in the butyrate treated cells compared to non-treated cells after 2.8mM and 16.7mM glucose stimulation, respectively.

Conclusion: Although higher intake of fiber-rich foods has been indicated to be beneficial for health including the risk of T2D, our results suggest that the risk increase of T2D by the TCF7L2 T-allele may be accentuated by higher fibers intakes. TCF7L2 activation by increased systemic butyrate levels and subsequent β -cell apoptosis could be a possible mechanism for our epidemiologic findings.

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A gene variant near ATM is significantly associated with metformin treatment response in type 2 diabetes: a replication and meta-analysis of two Dutch and two UK cohorts

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Background and aims: Recently we have demonstrated that the rs11212617 SNP near the ATM gene is significantly associated with metformin treatment response in type 2 diabetes patients from the UK. In this study we attempt to replicate our original observation in two additional cohorts from the Netherlands and perform a meta-analysis of all available replication cohorts.

Materials and methods: From the Diabetes Care System West-Friesland (DCS, n=929) and the Rotterdam study (n=182) in the Netherlands we selected incident users of metformin, either as mono therapy or in combination with stable sulphonylurea treatment. Subjects were included regardless of the baseline pre-treatment HbA_{1c} and all subjects were genotyped for rs11212617 (MAF=0.44) using standard techniques. Association between the SNP and treatment success, defined as the ability to reach the treatment target of an HbA_{1c} $\leq 7\%$ (53 mmol/mol) was tested using logistic regression with adjustment for potential confounders; baseline HbA_{1c}, baseline gap, drug adherence, daily dose, treatment group and creatinine clearance. Meta-analysis

was performed using the above described cohorts and two UK replication cohorts from our original publication (GoDARTS, $n=1965$; UKPDS, $n=1113$). Furthermore we performed a meta-analysis of the linear regression with the treatment HbA1c as continuous outcome.

Results: In the DCS cohort we observed a significant association between rs11212617 and treatment success on metformin ($OR=1.27$; 95% CI 1.03–1.58; $p=0.028$). In the smaller Rotterdam cohort a similar but non-significant trend was observed ($OR=1.45$; 95% CI 0.87–2.39; $p=0.15$). In a meta-analysis of all replication cohorts including in total 4189 subjects but excluding the original discovery cohort from the UK, this SNP is significantly associated with metformin treatment success ($OR=1.26$; 95% CI 1.14–1.39; $p=5.0 \times 10^{-6}$). The observed positive effect of this SNP was numerically greater in those on mono therapy compared to the dual therapy group ($OR_{mono}=1.32$; 95% CI 1.16–1.50 versus $OR_{dual}=1.23$; 95% CI 1.02–1.50). We also noted a significant association with treatment HbA1c in the meta-analysis (Beta -0.063 ; 95% CI -0.103 – -0.022 , $p=2.3 \times 10^{-3}$).

Conclusion: A gene variant near *ATM* is significantly associated with metformin treatment response in type 2 diabetes patients from the Netherlands and the UK. This is the first robustly replicated common susceptibility locus associated with metformin treatment response and our results warrant further genetic studies to identify other loci affecting diabetes treatment response.

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OP 29 Adipose tissue inflammation

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Adipose tissue alterations and liver disease in morbidly obese adolescent prior to bariatric surgery

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Background and aims: The growing epidemics of obesity affect adolescent and young adults. Early onset and severe obesity frequently persists at adult age. Therefore, obesity related conditions should be investigated early. The putative deterioration of adipose tissue (AT) or liver, two key tissues contributing to obesity related comorbidities are not well known in early onset obesity. We took advantage of a bariatric surgery programs in adolescent and young adults to explore liver and adipose tissue histopathology and compared them to adults with or without early onset obesity.

Materials and methods: After multidisciplinary and medical assessments, 15 adolescent (aged 19 ± 1.5 range [15–21]) were operated from bariatric surgery (group 1). We also chose two groups of 15 adults for comparison: group 2 was aged 47 ± 6 years with early onset obesity and group 3 was aged 52 ± 7 years with obesity starting after the age of 20. Group 2 had normal BMI at 20 years old (23.2 ± 1.7 kg/m²) whereas group 3 was already obese (BMI= 32.9 ± 4.5 kg/m²). All patients were evaluated for a series of bioclinical phenotypes. Importantly neither BMI nor body composition (DXA evaluated fat mass and fat free mass) differed among the three groups (48 ± 7 ; 46 ± 4 ; $\pm 43 \pm 6$ kg/m² respectively). During surgery, liver and paired surgical biopsies of subcutaneous (scWAT) and visceral (oWAT) adipose tissue were obtained. Adipocyte size and the number of HAM56 (a macrophage marker) positive cells normalized to 100 adipocytes was determined by immunohistochemistry.

Results: Histopathological evaluation of adipose tissue revealed that adolescent already had adipose tissue and liver alteration comparable to that of obese adults. We observed no difference in macrophage infiltration (scWAT $16.2 \pm 7\%$, oWAT= $19.4 \pm 6.2\%$) or adipocyte size (scWAT= 69.7 ± 13.3 , oWAT= 64.5 ± 13.4 μm) in the two sites of (AT) among the three groups. Regarding liver alterations in adolescent, 50% of the population presented steatosis (6 patients had grade I and 2 patients had grade II); 50% had fibrosis (6 patients scored 1 and 2 patients scored 2); none had overt NASH (Median NAS score was 1). Among adults in group 2, 50% of the population displayed steatosis (6 patients had grade III); 75% of this group had fibrosis (6 patients with stage 1 and 4 patients with stage 2); 20% had overt NASH (median NAS score of 3). Finally among group 3, 100% of the population presented steatosis grade II or III; 86 % of them had fibrosis (4 patients with stage 1, 5 patients with stage 2, 4 patients with stage 3); 33% of this group had overt NASH (Median NAS score was 4).

Conclusion: We demonstrate herein that young morbidly obese patients already have adipose and liver tissue perturbations. Their adipose tissue does not present any difference with that of adults presenting either early or latter onset obesity. Yet, both crown-like structures and macrophage phenotype (M2/M1) remain to be evaluated. On the contrary, our results suggest that liver alterations develop in a dose response manner to age and obesity duration.

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CCR5 deficiency attenuates obesity-induced insulin resistance through M2 dominant shift in adipose tissue macrophage

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Background and aims: Adipose tissue macrophage (ATM) accumulation through monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR2 is considered pivotal in the development of insulin resistance. However, deficiency of MCP-1 or CCR2 does not normalize obesity-induced ATM recruitment and insulin resistance. Therefore, other chemokine systems could

also play a role in these processes. Recently, we demonstrated that loss of a different C-C chemokine receptor, CCR5, prevents insulin resistance induced by high fat (HF) feeding or leptin deficiency independently of MCP-1-CCR2. In the present study, we show that CCR5 plays a crucial role in the inflammatory response to HF feeding and obesity by regulating both macrophage recruitment and M1/M2 status.

Materials and methods: We compared gene expression levels of CCR5 and its ligands in stromal vascular (SV) fraction to adipocyte fraction of epididymal white adipose tissue (eWAT) of genetically (ob/ob) and HF diet-induced obese (DIO) mice. To quantify CCR5⁺ ATMs in lean and obese mice, fluorescence-activated cell sorter (FACS) analysis was performed on SV cells from eWAT of HF diet-induced obese (DIO) mice. Next, we performed bone marrow transplantation (BMT) of CCR5^{-/-} mice or wild type (WT) C57BL/6J mice donor cells into irradiated either WT or ob/ob recipient mice to generate myeloid cell specific chimeric mice.

Results: Expression of mRNA for CCR5 and its all ligands (MIP-1 α , MIP-1 β , RANTES, MCP-2) was markedly increased in SV fraction compared to adipocyte fraction of eWAT of ob/ob and DIO mice. Immunohistochemistry on eWAT in DIO mice localized CCR5⁺ cells to F4/80⁺ macrophages in crown-like structure. FACS analysis revealed that ATMs identified as CD45⁺CD11b⁺F4/80⁺ cells were increased in DIO mice by 12.2-fold compared with WT mice. Only a small percentage of ATMs coexpressed CCR5 in WT mice. However, DIO mice had 11.9-fold increase in CCR5⁺ cells within ATMs, indicating that CCR5⁺ macrophages accumulate in eWAT of obese mice. On a chow diet, no differences were observed in either CD11c⁺ MGL1⁺ (M1) or CD11c⁺ MGL1⁺ (M2) expression within ATMs from WT and Ccr5^{-/-} mice. However, on a HF diet, in addition to reduction of total ATM content, Ccr5^{-/-} mice had 39% fewer M1 ATMs whereas and 33% more M2 ATMs than WT mice, resulting in predominance of M2 over M1 ATM population. Importantly, chimeric mice lacking CCR5 only in myeloid cells (BM transplant of Ccr5^{-/-} into WT) were protected from HF diet-induced hyperinsulinemia (BM transplant of Ccr5^{-/-} into WT 2.6 \pm 0.3 vs WT into WT 5.1 \pm 1.1 ng/ml, p <0.05) and glucose intolerance. Furthermore, ob/ob mice reconstituted with Ccr5^{-/-} myeloid cells were strikingly resistant to the development of insulin resistance and glucose intolerance compared to ob/ob mice reconstituted with Ccr5^{+/+} myeloid cells.

Conclusion: CCR5⁺ ATMs accumulate in obese mouse models. Deficiency of CCR5 causes M2 dominant phenotypic shift in ATM, which contributes to attenuation of obesity-induced insulin resistance. Therefore, CCR5 plays a critical role in obesity-induced adipose tissue inflammation and insulin resistance by regulating both macrophage recruitment and M1/M2 status.

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Visceral adipose tissue from obese diabetic patients has a stronger proliferative effect on smooth muscle cells and is characterised by higher expression and release of novel adipokines

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Background and aims: The accumulation of adipose tissue (AT) in obesity is an important risk factor for the development of metabolic syndrome. AT is known as a highly active endocrine organ, which is involved in many physiological processes by secreting adipokines. In a previous study, we characterized the secretome of *in vitro* differentiated human adipocytes using an unbiased proteomics approach, revealing novel adipokines like heme oxygenase-1 (HO-1), dipeptidyl peptidase-4 (DPP-4), factor H and alpha crystallin-B (CRYAB). The aim of this study is to characterize differential secretion and expression of these novel adipokines in visceral (VAT) and subcutaneous (SAT) AT of diabetic and non-diabetic patients and the impact of these various fat depots on the proliferation of human smooth muscle cells (SMC).

Materials and methods: SAT and VAT were obtained from lean ($n=14$, BMI 22.1 kg/m², mean age 65.9 yrs) and obese ($n=14$, BMI 34.7 kg/m², age 55.1 yrs) non-diabetics or lean ($n=5$, BMI 21.2 kg/m², age 70.8 yrs) and obese ($n=6$, BMI 36.7 kg/m², age 56.2 yrs) diabetics undergoing visceral surgery. Conditioned media (CM) were generated by using fat explants from paired SAT and VAT of each patient. VEGF and DPP-4 release were measured in CM by ELISA and protein level of novel adipokines in SAT and VAT were measured by Western Blot. Proliferation of primary human SMC treated with CM was measured by BrdU incorporation into DNA.

Results: The protein expression of CRYAB was significantly enhanced in VAT of obese non-diabetics (1.9-fold) as well as obese diabetics (1.6-fold)

compared to SAT of obese non-diabetics and diabetics. In contrast, there was no difference in CRYAB expression between fat depots of lean patients. Protein expression of factor H was elevated in VAT of lean and obese patients compared to their respective subcutaneous control. Expression of HO-1 was increased in VAT of obese non-diabetics (1.6-fold) as well as lean (1.8-fold) and obese (2.5-fold) diabetics compared to SAT of respective patients. In contrast to these novel adipokines, we also assessed adiponectin expression as a control and found decreased expression in VAT of lean and obese non-diabetics and diabetics as compared to SAT. Protein expression and secretion of DPP-4 was significantly enhanced in VAT of lean and obese patients with no differences between diabetics and non-diabetics. CM of VAT from obese non-diabetics significantly increased the proliferation of SMC (173.2 \pm 27.9 %) compared to SAT. Additionally, CM of SAT of obese diabetics induced a 1.5-fold increase of proliferation compared to SAT from lean diabetics. VAT from obese diabetics showed the strongest proliferative effect (233.5 \pm 17.5 %) compared to SAT of obese diabetics or VAT of lean non-diabetics. Proliferative effects were consistent with VEGF content of CM, which was significantly increased in VAT of obese non-diabetics (4.7-fold) and diabetics (6.1-fold).

Conclusion: This study revealed, that the protein level of novel adipokines, such as DPP-4, HO-1, factor H and CRYAB, was significantly increased in VAT from obese patients, especially obese diabetics. Furthermore VAT from obese diabetics had the strongest proliferative effect on SMC, indicating that the increased expression and release of these adipokines have an essential relevance linking obesity, diabetes and vascular dysfunction.

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Lack of SIRT1 leads to angiogenesis deficiency in adipose tissue through macrophage and adipocyte dysfunction

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Background and aims: SIRT1 (Mammalian sirtuins 1) is a class III histone deacetylase whose activation is dependent on NAD⁺ in cells. The biological activity of SIRT1 remains to be investigated in the regulation of microcirculation. Angiogenesis in adipose tissue is regulated by angiogenic factors from adipocytes and macrophages. However, the relative importance is unclear for adipocytes and macrophages. In this study, we addressed these issues by analysis of adipocytes and macrophages in adipose tissue of SIRT1^{-/-} and SIRT1^{+/+} mice.

Materials and methods: SIRT1^{-/-} mice on 129/J background were interbred with C57BL/6 mice for generations to get SIRT1^{+/+} mice with C57BL/6 background. Then SIRT1^{-/-} male and female mice on C57BL/6 gene background were set up breeding to get SIRT1^{-/-} mice. The assay was based on an adipose tissue imaging with specific dye under a confocal microscope. Reconstruction of 3D data sets was accomplished using Imaris.

Results: We observed that the density of vascular network was decreased by 50% in the tip portion of epididymal adipose tissue in the SIRT1^{-/-} mice, but no significant reduction in SIRT1^{+/+} mice. The cellular basis of decreased angiogenesis was investigated with a focus on macrophages and adipocytes. Macrophage function is impaired in the SIRT1^{-/-} mice. Macrophage number is significantly reduced in adipose tissue and their expression of angiogenic factors were reduced in SIRT1^{-/-} mice. When macrophage was deleted with liposome-encapsulated clodronate in the wild type C56BL/6J mice, the density of vascular network was decreased and this reduction was associated with loss of angiogenic factors (PDGF) expression. Meanwhile, adipocytes function is also impaired in the adipose tissue of SIRT1^{-/-} mice as adiponectin and leptin expression was reduced dramatically. VEGF, Agarnase1 and IL-6 were decreased significantly on differentiated MEF in SIRT1^{-/-} mice. However, no significant defects above were observed in SIRT1^{+/+} mice.

Conclusion: The data suggests that SIRT1 may regulate angiogenesis in adipose tissue through its activity in macrophage and adipocyte in a gene-dose dependant way. SIRT1 "gene dose" influences angiogenesis in adipose tissue. Angiogenic defect occurs in SIRT1^{-/-}, but not SIRT1^{+/+} mice. Deficiency of adipocytes and macrophages in angiogenic factor expression contributes to the angiogenic defect in SIRT1^{-/-} adipose tissue. The observation supports a new mechanism of SIRT1 action in the regulation of vascular growth.

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Immunosuppressive agents induce alterations in human subcutaneous adipose tissue gene expression and glucose and lipid metabolismM.J.R. Pereira^{1,2}, J. Palming¹, M. Rizell³, M. Gäbel³, M. Aureliano², E. Carvalho⁴, M.K. Svensson⁵, J.W. Eriksson⁶;¹The Lundberg Laboratory for Diabetes Research, University of Gothenburg, Sweden, ²Center for Marine Sciences, Faro, Portugal, ³Department of Surgery, University Hospital of Gothenburg, Sweden, ⁴CNC, University of Coimbra, Portugal, ⁵Department of Molecular and Clinical Medicine, University Hospital of Gothenburg, ⁶AstraZeneca R&D, Mölndal, Sweden.

Background and aims: Immunosuppressive agents (IA), such as cyclosporine (CsA), tacrolimus (FK) and rapamycin (Rap) can cause new-onset diabetes (NODAT) as well as dyslipidemia in solid organ-transplantation patients. The studies of the effects of IA in human adipocytes are few, and it is believed that it could be a good model to understand the effects of IA in humans. The aim of this study was to investigate whether IA could cause alterations on glucose and lipid metabolism as well as on gene expression in human subcutaneous adipose tissue.

Materials and methods: Abdominal subcutaneous adipose tissue was obtained from surgical and needle biopsies from healthy volunteers (24F/15M, 23–72 yrs, BMI: 21–36 kg/m²). Adipose tissue and isolated adipocytes were incubated in the absence and presence of IA: CsA and FK (0.1 µM) and Rap (0.01 µM). Basal and insulin-stimulated glucose uptake (n=18), lipolysis (n=13), signalling protein amount and phosphorylation (30 min, 3h and 20h, n=6) and gene expression (n=13) were assessed.

Results: It was observed that all three IA inhibited both basal: CsA 28%, FK 30% and Rap 18% (p<0.05) and insulin-stimulated: CsA 27%, FK 28% and Rap 26% (p<0.001) 14C-glucose uptake compared to controls. Rap decreased IRS2 protein levels and insulin-stimulated phosphorylation of PKB Ser473 by 20% (p<0.05) compared to control. A reduction in rictor-mTOR interaction was found after Rap treatment (35%, p=0.04). Following CsA and FK incubation the total expression and phosphorylation of initial proteins of the insulin signaling cascade: IR/IRS1-2/PKB/AS160, were not changed. Also, the protein level of GLUT4, or its gene expression was not changed, upon exposition to any of the IA. CsA, FK and Rap increased isoprenaline-stimulated lipolysis (30% p<0.01; 20% p<0.05 and 20% p<0.05, respectively) compared to control, but only Rap increased basal lipolysis (40% p<0.05). In addition, treatment with Rap was associated with increased perilipin and IL6 and a decrease of lipin1, leptin and the inflammatory mediator TNF-alpha gene expression (p<0.05) compared to control. Paradoxically, IRS2 gene expression was increased after Rap treatment (p<0.05). No effects of IA on IRS1, LPL, HSL and adiponectin gene expression were observed.

Conclusion: It was demonstrated for the first time that in human adipocytes CsA and FK at therapeutic concentrations inhibits both basal and insulin-stimulated glucose uptake, but did not change the initial steps of the insulin signaling cascade or GLUT4. Moreover, Rap may impair insulin action on glucose uptake by reducing IRS2 protein levels and AKT Ser473 phosphorylation in the human subcutaneous adipose tissue. All three IAs increased lipolysis but only Rap was found to alter gene expression of important lipolysis regulators and adipokines that may induce insulin resistance. These observed effects of IA in the adipose tissue could potentially contribute to the development of new-onset diabetes and dyslipidemia in solid organ-transplant patients.

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Role of lipokines and oxysterols as novel adipose-derived lipid “hormones” linking adipose (dys)function and insulin resistanceG. Murdolo¹, C. Tortoioli¹, M. Piroddi¹, B. Canonico², F. Luchetti², L. Iuliano³, F. Galli¹;¹Dpt Internal Medicine, Perugia University, ²Dpt. of Earth, Life and Environmental, University of Urbino Carlo Bo, ³Dpt. of Medical Sciences and Biotechnology, Unit of Vascular Medicine, Sapienza University of Rome, Italy.

Background and aims: Experimental data suggest that lipokines (adipose-derived “lipid-hormones”) and oxysterols (oxidized derivatives of cholesterol) may play an important role in linking the adipose organ (dys)function with impaired glucose homeostasis. Yet, the role of these lipid hormones/oxi-

dation derivatives in human adipose tissue (AT) remains largely unexplored. Here we combined a “lipidomic” approach (gas chromatography-mass spectrometry) with the subcutaneous (SC) microdialysis technique to characterize the adipose-derived profile of fatty acids (FA) and oxysterols *in vivo*. Also, we tested the hypothesis that 7-ketocholesterol (7κ-C) and 4-hydroxynonenal (4-HNE), the more representative oxidation products of cholesterol and polyunsaturated fatty acids, respectively, may modulate the lineage-specific differentiation of adipose-derived stem cells (ASC).

Materials and methods: Abdominal SC, mesenteric (MES) and omental (OM) fat specimens were obtained from obese nondiabetic (OB) and type 2 diabetic (OBT2D) patients. Flow cytometry and western blot analysis were performed on isolated ASC and whole tissue specimens for cell sorting and oxidative stress evaluation, respectively.

Results: In AT interstitial fluid, abundant concentrations of oxysterols (ie, 7κ-C) and lipokines were found. The major FA in dialysate were palmitic (16:0, 27%), stearic (18:0, 23%), elaidic (18:1 *n*-9*t*, 20%) and linoleic (18:2 *n*-6, 14%) acid, which made about 85% of total FA. The profile of FA in plasma, showed higher oleic (18:1 *n*-9, 22 vs 1.4%) and linoleic (26 vs 14%), lower stearic (8 vs 23%) and similar palmitic (22 vs 27%) and palmitoleic (16:1, 1.6 vs 1.8) acid, as compared with interstitial fluid. The immunophenotype of isolated ASC revealed positivity for CD29, CD44, CD71, CD90 and CD106 (mesenchymal stem cell markers), with slight CD34 (endothelial stem cell marker), but negative CD135, CD11b, CD45 and HLA-DR (leukocyte and macrophage markers) expression. After incubation with 7κ-C at 10–100 µM, CD29/CD106/CD34-positive precursor cells from the SC, MES and OM fat depot showed a dose-dependent decrease in cell viability, while at levels resembling those found in dialysate (>1<10 µM) 7κ-C challenge virtually abolished adipogenic differentiation without affecting cell viability. In OBT2D patients, adipogenic differentiation (Oil Red O) of the SC precursor cells was further impaired, as compared with that of ASC from OB individuals. Accordingly, in the SC fat of OBT2D patients, the expression of 4-HNE was meaningfully increased when compared with that of OB subjects that, in turn, displayed more abundant 4-HNE expression in OM and MES than in SC fat depot, respectively. Finally, activation of the WNT-signaling pathway by 7κ-C and 4-HNE is under investigation.

Conclusion: Human AT emerges as a critical compartment for storage and secretion of lipokines and oxysterols, which, when in excess, may detrimentally affect the adipogenic differentiation of ASC. We thus suggest the adipose-derived lipid hormones and oxidized cholesterol derivatives as new players involved in adipose organ dysregulation and “diabetic” adiposopathy. Supported by: Fondazione Cassa di risparmio di Perugia

OP 30 Neuropathy: experimental and clinical

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Alterations in the corneal nerve plexus of diabetic NOD mice

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Background and aims: Macro- and microangiopathy are serious long-term consequences of diabetes mellitus. Patients with type 1 diabetes mellitus have particularly a high risk to develop neuropathy. Engineering of in-vivo confocal laser scanning microscopy in recent years facilitate for the first time examination of the human cornea. Implementation of this innovative technique has provided direct evidence for distinct changes of the corneal nerves due to diabetes mellitus in humans. This raised the question whether corneal neuropathy directly correlates with hyperglycaemia. The NOD mouse is a well characterized animal model of type 1 diabetes mellitus. Therefore the aim of this study was to establish in-vivo visualization of corneal nerves in NOD mice by confocal laser scanning microscopy and to compare corneal innervations in diabetic and healthy animals.

Materials and methods: NOD mice were characterized by measuring auto-antibodies against insulin, blood glucose and HbA1c. For confocal laser scanning microscopy the animals were anesthetized and fixed to avoid movement. The eye was moistened with a carbomer gel and the cornea was visualized by single image acquisition or a z-layer image sequence by confocal laser scanning microscopy (HRTII + RCM, Heidelberg Engineering). Thereafter images were analysed using AutoQuant/AutoDeblur Software (Media Cybernetics).

Results: NOD mice with a high level of auto-antibodies against insulin at the age of 20 weeks were classified as diabetic. These animals had with 24 mmol/l and 10.5 % a significant higher blood glucose level and HbA1c value, respectively in comparison with healthy animals (5 mmol/l and 4%). Five healthy and diabetic mice each were examined by corneal confocal laser scanning microscopy. Quantification of the total number of nerves per image frame revealed a significant reduction in sub-basal nerves in diabetic NOD mice compared with their healthy littermates. Furthermore a reduction in the homogeneous linear texture of the nerves was observed in diabetic NOD mice. Deconvolution of a z-layer image sequence revealed a beaded structure of nerve bundles in healthy mice in a comparable manner as previously described in humans.

Conclusion: We could demonstrate for the first time in-vivo confocal laser scanning microscopy of mouse corneal nerves in healthy and diabetic NOD mice. It will be the challenge of a comprehensive study to elucidate correlations of hyperglycaemia and alterations in corneal nerves in terms of time response, reversibility and protection. This will pave the way to establish this non-invasive technique as an early diagnostic criterion of diabetes mellitus.

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Autonomic neuropathy depresses circulating angiogenic cells

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Background and aims: Diabetic patients have reduced levels of circulating endothelial progenitor cells (EPCs), which may contribute to vascular complications. The mechanisms accounting for reduced EPCs in diabetes are incompletely understood and include a defective bone marrow mobilization. Experimental data indicate that bone marrow autonomic neuropathy hampers mobilization of EPCs to the bloodstream and precedes the development of retinopathy. We aimed to assess the association and causal relationship between cardiac autonomic neuropathy (CAN) in diabetes.

Materials and methods: We have enrolled 65 diabetic patients in an observational study. We determined the level of circulating CD34+ angiogenic cells. We collected anthropometric data and information on concomitant risk factors and diabetic complications. The patients were subjected to 4 CAN tests: orthostatic hypotension; valsalva maneuver; lying-to-standing; deep breathing. Patients were divided according to the number of altered CAN tests. Age specific threshold were used. To understand the causal relationship between autonomic denervation and alteration in angiogenic cells, we measured

CD34+Flk1+ endothelial progenitor cells (EPCs) in wild type C57Bl/6 mice (n=4) before and after treatment with the gangliotoxin 6-hydroxy-DOPA (6-OH-DOPA).

Results: Out of 65 diabetic patients included in the study, 39 had normal CAN tests, 14 had one pathologic CAN test and 12 had at least two pathologic CAN tests. Patients with 2+ pathologic CAN tests had a significant 40% reduction of circulating CD34+ cells (p=0.002). A linear inverse correlation was found between number of altered CAN tests and CD34+ cell level (r=-0.38; p<0.001). Significant correlations were also found between CD34+ cells and single CAN test indexes (deep breathing: r=0.32; p=0.006; lying-to-standing: r=0.41; p<0.001; Valsalva maneuver: r=0.34; p=0.003; orthostatic hypotension: r=-0.29; p=0.014). In mice, the level of circulating EPCs was significantly reduced by about 50% after pharmacologic induction of experimental autonomic neuropathy (35.6±15.5 vs 19.3±9.20 cells/millions of events, control vs 6-OH-DOPA, p=0.005).

Conclusion: Autonomic neuropathy in diabetic patients is strongly associated with depletion of circulating angiogenic cells. According to preliminary data in mice, this association may have a causal relationship.

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Evaluation of the Neuropad[®] sudomotor function test as a screening tool for polyneuropathy in the elderly population with diabetes and prediabetes: the KORA F4 survey

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Background and aims: Neuropad[®] is a new indicator test for sudomotor dysfunction. This study aimed to examine the sensitivity and specificity of Neuropad[®] as a screening tool for distal symmetric polyneuropathy (DSPN) among elderly subjects with diabetes and pre-diabetes in the general population.

Materials and methods: The population-based KORA (Cooperative Health Research in the Region of Augsburg) S4 survey (1999–2001) included 2656 subjects aged 55–74 years, who were living in the region of Augsburg, Germany. Of these, 1653 participated and 1353 subjects without known diabetes had an oral glucose tolerance test (OGTT) at baseline. This cohort was re-examined between 2006 and 2008 (F4 survey) including 1129 subjects aged 61–82 years (mean±SD: 69.8±6.5 years). Of these, 940 subjects were available for re-examination after 189 subjects had been excluded due to relevant neurological and musculoskeletal diseases. Based on the previous diagnosis of diabetes or OGTT results in F4, 201 subjects had diabetes (148 with known diabetes, 53 with diabetes detected by OGTT) and 231 had pre-diabetes (WHO 1999 criteria). Neuropad[®] was applied on a callus-free area between the 1st and 2nd metatarsal head on the plantar aspect of the foot and the result was read at 10 minutes (normal: complete colour change from blue to pink; abnormal: no/incomplete colour change). DSPN was diagnosed by clinical neurological examination using the Michigan Neuropathy Screening Instrument (MNSI) and defined as an MNSI score >2.

Results: DSPN was diagnosed in 74 (36.8%) subjects with diabetes and in 72 (31.2%) persons with pre-diabetes, respectively. The sensitivity and negative predictive value (NPV) of Neuropad[®] for the diagnosis of DSPN were moderately high, reaching 75.7% and 71.9% in subjects with diabetes and 68.1% and 71.6% in those with pre-diabetes, respectively. In contrast, the specificity and positive predictive value (PPV) for the diagnosis of DSPN were relatively low, yielding only 36.2% and 40.9% in diabetic individuals and 36.5% and 32.7% in persons with pre-diabetes, respectively.

Conclusion: In the elderly general population, Neuropad[®] has reasonable sensitivity but rather low specificity for the diagnosis of DSPN. It is a useful simple and inexpensive tool to screen for and to exclude DSPN as desired, while its low specificity implies that a longer reading time merits consideration in older subjects with diabetes and pre-diabetes.

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The modified LDI Flare technique demonstrates sub maximal stimulation of C-fibres with current methods and reveals abnormal C-fibre function in individuals with diabetes

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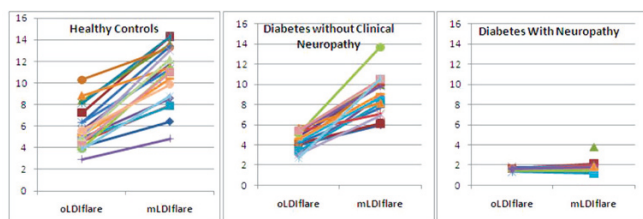
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Background and aims: Diabetic peripheral neuropathy (DPN) can affect at least 50% of individuals with diabetes and results in significant morbidity, mortality and healthcare costs. There is increasing evidence that small unmyelinated fibres which mediate pain and temperature (C-fibres) are damaged in early diabetes before clinical signs or electrophysiological changes arise. We have developed a non-invasive method of assessing C-fibre function involving skin heating to 44°C using a 1cm² probe and measuring the resultant flare area (LDIflare). We had chosen this temperature as temperatures between 42–44°C have been shown to stimulate maximal hyperaemia (LDImax), a marker of endothelial function, and therefore, we assumed were likely to also maximally stimulate C-fibre activation. In this study we have challenged our assumption by studying subjects at 44°C and 47°C. The latter method we have termed the modified LDIflare (mLDIflare) and the previous method the original LDIflare (oLDIflare).

Materials and methods: Our first study was in a group of healthy controls (HC, n=18, males=7 aged 40.3±11.2 years (mean±SD). Our second study included a group of individuals with diabetes and without clinical neuropathy (DN-, n=18) and a group with clinical neuropathy (DN+, n=10) and compared them to findings in HC. Mann-Whitney U test was used to compare the variables.

Results: In the first study of HC, LDImax was not different in the two methods (oLDImax= 811±179 PU, mLDImax = 845 ±157 PU, P=NS). In contrast mLDIflare produced a significantly bigger flare response (oLDIflare = 5.79±1.9 cm², mLDIflare = 11.2± 2.66 cm² (P<0.0001). Correlation between the two methods was r=0.81, P<0.0001). In the second study in DN- subjects the oLDIflare was significantly smaller compared with HC and DN-subjects (oLDIflare area: DN- = 4.44±0.93 cm² v HC=5.79±1.9 cm², and DN+ 1.58±0.15 cm²; P<0.0001 and P=0.01, respectively (Figure 1). The corresponding mLDIflares in DN- = 8.95±1.88 cm² (P<0.0001 compared to oLDIflare), and DN+ = 1.88±0.7 cm² p=NS compared to oLDIflare). The percentage increase in flare area in DN- was similar to HC (109±62% and 92±41% respectively, p=0.39) but was significantly lower in DN+ (10.5±12.1%, p<0.0001).

Conclusion: These results confirm that in healthy individuals 44°C induces maximal vasodilatation, and demonstrate that C-fibres are submaximally stimulated at this temperature. They also suggest that the nerve axon reflex pathway is an independent measure of C-fibre function and is independent of endothelial function. Although individuals with diabetes without neuropathy have impaired neurovascular responses they are able to augment C-fibre function similar to healthy controls, however this does not occur in the presence of clinical neuropathy.

Fig 1. Comparative oLDIflare and mLDIflare areas (cm²) in the 3 groups studied.

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The neural correlates of central pain processing in diabetic neuropathy - a functional magnetic resonance imaging study

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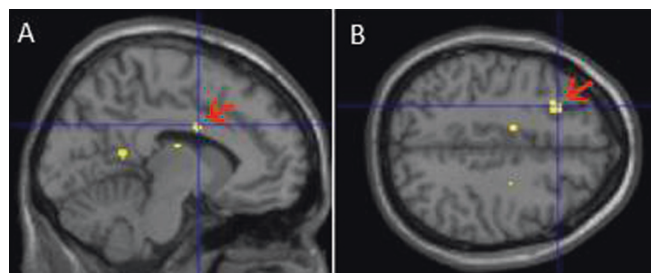
Background and aims: Patients with painful diabetic neuropathy (DN) suffer with distressing and disabling symptoms. Unfortunately, current treatments for painful-DN are inadequate, partly as the development of new analgesics has been hindered by the poor translation of their efficacy from

animal studies to the human condition. There is thus a clear need to develop objective biomarkers of neuropathic pain in order to evaluate the efficacy of new therapies. The aim of this study was to investigate the brain's responses to acute painful stimuli to identify the neural correlates of central nociceptive processing in DN.

Materials and methods: 34 age and sex matched groups type 1 diabetes subjects (12 no-DN, 11 painful-DN and 11 painless-DN), 12 healthy volunteers (HV) and 12 disease control subjects with chemotherapy induced painful neuropathy (CIPN) underwent functional magnetic resonance imaging during noxious heat versus pain-free baseline thermal stimulation in a 'boxcar' paradigm. This was performed on the neuropathic site (dorsum of foot) and non-neuropathic site (anterior thigh). Images were analysed using SPM (www.fil.ion.ucl.ac.uk/SPM). Prior to scanning all subjects had detailed characterisation of peripheral neuropathy [NIS(LL)+7 tests] and pain phenotyping. Painful neuropathy subjects had severe neuropathic pain (Likert scale NRS > 4) below the knees.

Results: Painful stimuli delivered to the neuropathic foot resulted in significantly greater activation in the dorsolateral prefrontal cortex (DLPFC, Talairach coordinates -8, 0, 31mm, p<0.001 uncorrected; Figure B) and cingulate gyrus (-8,0,31mm, p<0.001; Figure A) in painful-DN compared to HV. Increased activation in these regions persisted during nociceptive stimulation of the non-neuropathic thigh location in painful-DN compared with painless-DN. In contrast, when compared to HV, CIPN subjects did not demonstrate a similar pattern of neuronal activation as seen in painful-DN. Subjects with painful-DN had greater activation of the paracentral gyrus (-10,-12,44mm, p<0.001) when compared with CIPN.

Conclusion: The prefrontal cortex is involved in processing of emotions and mood. Increased activation reflects the cognitive set of painful-DN with high prevalence of mood disorders. The cingulate gyrus processes the 'suffering' component of pain. Increased activation suggests greater emphasis on the affective dimension in acute pain processing in painful-DN not seen in the disease control group. The paracentral gyrus is involved in processing sensory signals from the lower limb. Together with the cingulate gyrus, these two regions may serve as objective neural correlates of pain in DN. Further validation studies are required to examine the utility of this technique to evaluate the efficacy of new therapies.



Supported by: JDRF

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Predictors of peripheral neuropathy and effects of fenofibrate among 9,795 subjects with type 2 diabetes: the fenofibrate intervention and event lowering in diabetes (FIELD) study

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Background and aims: Peripheral neuropathy, a significant microvascular complication of diabetes, leads to impaired quality of life, and lower limb ulcer formation and amputation. The aim of this analysis was to explore characteristics associated with neuropathy and the effects on it of long-term treatment with the lipid-modifying drug, fenofibrate.

Materials and methods: 9,795 patients with type 2 diabetes mellitus were randomised to co-micronised fenofibrate 200 mg/day or matching placebo for 5 years. The presence of neuropathy symptoms was recorded at baseline and sensation tested using a standard 10-G monofilament, repeated at 2 years and study close. All non-traumatic lower limb amputations were recorded, and classified as major or minor. Risk models used exhaustive search methods in logistic regression.

Results: 17.2% (1689 of 9795) of participants had features of peripheral neuropathy at baseline (1125 had symptoms without abnormal monofilament;

564 had abnormal monofilament). Factors associated with onset of new neuropathy included age, diabetes duration, high HbA_{1c}, prior retinopathy, hypertriglyceridaemia, history of CVD, and height (all $p < 0.03$). The prevalence of an abnormal monofilament test at study close was significantly lower among those allocated to receive fenofibrate than placebo (6.9% versus 8.2%; RR 0.81 [95%CI 0.72–0.93, $p = 0.002$ adjusted for baseline, with significantly less new neuropathy with fenofibrate treatment, and significant reversal of baseline monofilament abnormality with treatment (RR=1.28, [95% CI 1.06–1.55], $p = 0.01$). Neuropathy was one of the strongest predictors of amputation, increasing the risk of a first amputation approximately sixteen-fold for those with both symptoms and an abnormal monofilament test at baseline ($P < 0.001$) in whom the number needed to treat (NNT) over 5 years to avoid a first on-study amputation with fenofibrate treatment was only 16.

Conclusion: Treatment with fenofibrate reduced overall monofilament abnormality as a marker of neuropathy and increased the reversal of pre-existing monofilament abnormality in type 2 diabetes, although the mechanisms remain unknown. These benefits could potentially explain the large 37% reduction seen in amputations with fenofibrate use in the FIELD study.

Clinical Trial Registration Number: ISRCTN64783481

Supported by: Abbott Laboratories

OP 31 Metabolic effect of bariatric surgery

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Effect of Roux-En-Y gastric bypass on beta cell function and glucose metabolism in type 2 diabetic and non-diabetic patients

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Background and aims: Roux-en-Y gastric bypass (RYGB) surgery leads to remission of diabetes (T2D) in a high percentage of cases; the mechanisms are not completely understood. While recent data suggest an effect of RYGB on beta cell function in T2D patients early after surgery, the long-term effects have not been investigated.

Materials and methods: We measured the metabolic response to a mixed meal (MMT) in 16 morbidly obese non-diabetic (M-Ob) and 13 morbidly obese T2D patients before (BMI=52±1 and 50±2 kg/m², respectively) and one year after RYGB (BMI=36±2 and 33±2, respectively, $p < 0.0001$); 7 normal-weight subjects (Ct, BMI=23±1) and 14 obese volunteers BMI-matched to postsurgery patients (Ob, BMI=34±1) served as controls. MMT was combined with the double tracer technique and mathematical modelling to measure β -cell glucose sensitivity (β -GS), rate of oral glucose appearance (RaO) and endogenous glucose production (EGP).

Results: In T2D, surgery normalised fasting plasma glucose (8.6±0.8 to 5.3±0.2 mM, $p = 0.005$) and HbA_{1c} levels (7.3±0.5 to 5.4±0.1%, $p < 0.01$). On the MMT, the postprandial plasma glucose and insulin secretion patterns were similar in T2D and M-Ob, with a rapid increase after ingestion of the meal followed by a sharp drop. In M-Ob, β -GS decreased slightly (from 125[63] to 91[19] pmol·min⁻¹·m⁻²·mM⁻¹, median[IQR], $p = 0.02$), but was still within the normal range ($p = \text{ns}$ vs Ct or Ob); in T2D, β -GS doubled (from 33[29] to 62[54] pmol·min⁻¹·m⁻²·mM⁻¹, $p = 0.05$) but did not normalize ($p < 0.05$ vs Ct or Ob). RaO patterns were similar in T2D and M-Ob, with a rapid increase after meal ingestion followed by a sharp drop, indicating rapid absorption of glucose; RaO area-under-curve was unchanged. In both surgical groups, the EGP time-course was the mirror image of Rao, the drop in Rao being synchronous with an increase in EGP. EGP area-under-curve increased in both M-Ob (from 1.57[0.80] to 2.1 [0.89] mmol·kg_{FEM}⁻¹·h⁻¹, $p = 0.008$) and T2D (from 1.38[1.22] to 2.26[1.4], $p = 0.05$) and in both was higher than in Ob or Ct ($p = 0.02$ for both). In both M-Ob and T2D, fasting plasma glucagon levels decreased but post-meal glucagon excursions were accentuated post-surgery.

Conclusion: Following RYGB-induced major weight loss, the glycaemic response to a mixed meal shows a biphasic, dumping-like, time-course, which is associated with augmented EGP and hyperglucagonaemia, presumably to counter late post-meal hypoglycaemia.

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Improved glucose metabolism early after gastric bypass surgery relies primarily on enhanced insulin sensitivity

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Background and aims: Gastric bypass surgery has been shown to resolve type 2 diabetes but the underlying mechanisms are incompletely understood. The aim of the study was to quantify changes in beta cell function and insulin sensitivity before and shortly after gastric bypass in type 2 diabetic (DM) patients and non-diabetic patients (non-DM) with severe obesity.

Materials and methods: Before and 8 to 21 days after the surgery, 20 DM patients (10 patients with diabetes duration ≥ 9 years) and 8 non-diabetic patients underwent an oral glucose tolerance test (OGTT) and a botnia clamp which combines an IVGTT with a subsequent hyperinsulinaemic-euglycaemic clamp. Established models were used to calculate various indices of glucose metabolism. During OGTT, glucagon-like peptide 1 (GLP-1) and glucagon time profiles were assessed.

Results: After surgery, a comparable decrease in body weight and BMI was achieved in the DM and non-DM group. HbA_{1c}, fasting glucose and 2h glucose response in the OGTT declined after surgery in DM (all $p < 0.05$). DM patients with a disease duration of ≥ 9 years showed a greater glucose AUC during OGTT than patients with a shorter diabetes duration before as well as after surgery (both $p < 0.05$) but a comparable improvement induced by the surgery. Among the OGTT insulin secretion indices, only the ratio AUC C-peptide/AUC glucose pointed to slightly improved insulin secretion in the DM patients (pre- vs. postoperative 0.37 ± 0.06 vs. 0.58 ± 0.08 ; $p < 0.01$), which overall remained substantially impaired when compared to the non-DM patients (pre- vs. postoperative 1.21 ± 0.06 vs. 1.44 ± 0.07). None of the insulin secretion indices differed between patients with a long or short diabetes duration. Indices of insulin sensitivity showed a marked improvement in DM patients (e.g. glucose disposal: 2.34 ± 0.42 vs. 4.11 ± 0.35 mg·kg⁻¹·min⁻¹; $p < 0.001$) which did not depend on the disease duration, whereas no such postoperative changes were observed in non-DM group. Plasma GLP-1 and glucagon concentrations showed a distinctly greater increase after the oral glucose load after than before surgery independently of the DM state (all $p < 0.05$).

Conclusion: The improvement in insulin sensitivity appears to be the main factor underlying the improvement in glucose metabolism shortly after gastric bypass surgery. Despite of a markedly enhanced GLP-1 response in OGTT there was only a mild improvement in insulin secretion which still remained impaired in comparison to non-DM patients. The unexpected enhanced rise in glucagon levels during the OGTT points to a substantial stimulatory effect of gastric bypass on alpha cell function.

Clinical Trial Registration Number: NCT01271062

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Enhanced myocardial glucose uptake after bariatric surgery in morbidly obese subjects

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Background and aims: Obesity and type 2 diabetes are associated with higher uptake and oxidation of fatty acids and insulin resistance, but insulin stimulated glucose uptake (GU) of the heart has been normal in patients with moderate obesity. The aim of this study was to investigate heart metabolism and function in morbidly obese subjects and evaluate the effects of bariatric surgery-induced weight loss in adult obese non-diabetic and diabetic patients.

Materials and methods: Eight non-diabetic (BMI 42.6 ± 3.5 kg/m²) and 15 diabetic or pre-diabetic obese patients (BMI 44.1 ± 4.1 kg/m²) were studied before and six months after a bariatric surgery and compared to ten healthy volunteers (BMI 23.7 ± 1.8 kg/m²). Heart GU was measured with fluorine 18-2-fluoro-2-deoxyglucose and positron emission tomography during euglycemic hyperinsulinemic clamp. Myocardial triglyceride content, cardiac structure and function were assessed with magnetic resonance imaging and magnetic resonance spectroscopy.

Results: Body weight and BMI decreased similarly after the bariatric surgery in the non-diabetic and the diabetic group (BMI - 20 ± 6 % in non-diabetics and - 25 ± 6 % in diabetics, $p < 0.001$ vs. baseline). OGTT was normalized in 10 out of 13 patients after the follow-up. Heart GU increased after the surgery (from 23.5 ± 8.9 to 34.6 ± 11.0 $\mu\text{mol}/100\text{g}/\text{min}$ in non-diabetic, $p = 0.02$, and from 23.4 ± 13.0 to 32.0 ± 10.1 $\mu\text{mol}/100\text{g}/\text{min}$, $p = 0.01$, in diabetic group). No group differences were observed in heart GU or myocardial triglyceride content before or after the bariatric surgery. Whole-body insulin mediated glucose uptake (M-value) was significantly impaired in both groups (non-diabetic 14.0 ± 7.0 and diabetic 11.8 ± 5.2 $\mu\text{mol}/\text{min}/\text{kg}$) and improved post-operatively by 81 ± 48 % and 119 ± 92 %, ($p < 0.001$ and $p = 0.001$, respectively) still remaining significantly lower compared to healthy controls (40.3 ± 9.5 $\mu\text{mol}/\text{min}/\text{kg}$) (both groups, $p < 0.001$). Heart GU tended to correlate with plasma free fatty acid concentration preoperatively ($p = 0.06$) but not with body adiposity, myocardial triglyceride content or M-value. The results of myocardial structure and function are under evaluation.

Conclusion: These results show that morbidly obese subjects have significant myocardial insulin resistance, which is improved similarly in morbidly obese non-diabetic and diabetic patients after bariatric surgery.

Clinical Trial Registration Number: NCT00793143

Supported by: EU FP6 (18734, HEPADIP), Academy of Finland (CoE), Sigrid Juselius foundation

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Short-term and longer-term metabolic effects of biliopancreatic diversion (BPD) in non-morbidly obese patients with type 2 diabetes mellitus

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Introduction and aim: Bariatric surgery is known to lead to remission of T2DM in a large proportion of morbidly obese patients. Objective of this study was to investigate the metabolic effects of BPD in non-morbidly obese T2DM.

Subjects and methods: We studied 16 patients (56 ± 4 years, BMI range $26.9 - 33.1$ kg/m²) with T2DM (duration 15 ± 6 years, all on treatment with metformin+sulfonylureas and 10 also on insulin [mean dose = 30 IU/day] before, 2 months, and 1 year after BPD. Each study consisted of a euglycemic hyperinsulinemic (240 pmol·m⁻²·min⁻¹) clamp, a standard OGTT, and a 5-hour mixed meal (561 kcal: protein 16%, lipid 30%, carbohydrate 54%) test.

Results: Two months after surgery, BMI had decreased from 28.1 ± 2.3 to 24.6 ± 2.1 kg/m² ($p < 0.001$); at one year, BMI was 23.2 ± 1.8 ($p = 0.002$ vs baseline). HbA_{1c} decreased from 8.6 ± 1.3 % to 6.7 ± 0.9 % at 2 months to 6.0 ± 1.0 % at 1 year ($p = 0.0004$ and $p = 0.002$ respectively). Fasting plasma glucose dropped from 12.3 ± 1.8 to 8.4 ± 2.6 , and to 7.7 ± 2.1 mmol/l ($p = 0.05$ and $p = 0.01$ at 2 months and 1 year, respectively); 2-hour post OGTT plasma glucose levels fell from 22.1 ± 2.5 to 12.7 ± 3.1 to 12.6 ± 3.1 mmol/l ($p < 0.001$ for the trend). At 2 months, no patient was on oral antidiabetic treatment, 8 were on insulin alone (mean dose = 14 IU/day); at 1 year, 6 patients were on insulin alone (mean dose = 12 IU/day). Insulin sensitivity (as the M value) increased from 20.1 ± 3.4 $\mu\text{mol}/\text{min}^{-1}\text{kg}_{\text{FFM}}^{-1}$ at baseline to 34.2 ± 11.2 at 2 months ($p = 0.0007$). The latter value is significantly less than in 60 age- and BMI-matched nondiabetic controls (58.5 ± 22.9 $\mu\text{mol}/\text{min}^{-1}\text{kg}_{\text{FFM}}^{-1}$, $p < 0.001$). The M value at 1 year was unchanged vs 2 months (33.1 ± 4.1 , $p = \text{ns}$). On the mixed meal, the ratio of C-peptide to glucose incremental areas rose from 150 ± 55 to 298 pmol/mmol ($p < 0.01$), indicating a $\sim 100\%$ increase in β -cell response.

Conclusion: In non-morbidly obese patients with long-standing, poorly controlled T2DM, BPD is followed by a substantial amelioration in glycemic control due to major improvements in both insulin sensitivity and β -cell function despite relatively small weight loss. The improvement is evident early after surgery and is maintained in the longer term. Defects in both insulin sensitivity and β -cell, however, persist at one year.

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Intestinal insulin resistance associated with obesity and type 2 diabetes

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Background and aims: The role of the proximal intestine in the pathophysiology of type 2 diabetes mellitus has been subject to growing interest. The remarkable results of bariatric surgery in improving glucose tolerance in obese diabetics are not still fully explained. We hypothesized that intestinal insulin resistance is an important factor in the pathophysiology of DM2. Our aim was to quantitate non-invasively intestinal insulin-stimulated glucose uptake (GU) in obese before and after bariatric surgery.

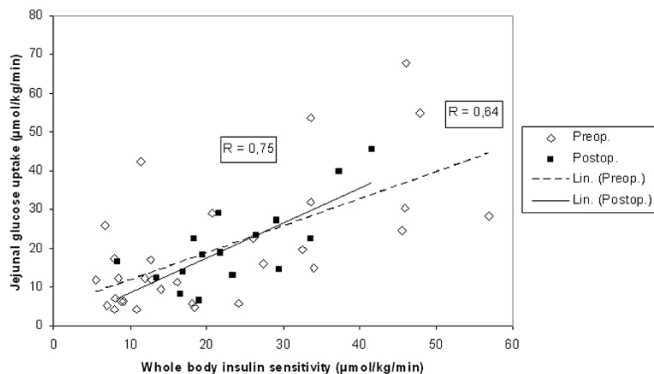
Materials and methods: We recruited 21 obese patients (age 47.3 ± 9.4 years, BMI 43.2 ± 3.8 kg/m²) of whom 13 had DM2 or impaired glucose tolerance and 10 age-matched healthy subjects. (BMI 23.7 ± 1.8 kg/m²). Patients were studied before and six months after bariatric surgery (Roux-en-Y gastric bypass or sleeve gastrectomy). Skeletal muscle and intestinal GU were studied using [¹⁸F]fluoro-2-deoxyglucose and positron emission tomography (PET) during euglycemic hyperinsulinemia. Intestinal GU was measured in the descending duodenum, the jejunum and the colon using graphical analyses and MRI fusion images were used as the anatomical reference.

Results: Both duodenal (17.8 ± 12.1 vs. 34.3 ± 13.4 $\mu\text{mol}/\text{kg}/\text{min}$) and jejunal glucose uptake (12.6 ± 9.7 vs. 34.5 ± 17.6 $\mu\text{mol}/\text{kg}/\text{min}$) were significantly lower ($p < 0.005$) in the obese compared to the lean group, but no difference was found in ascending colon. Bariatric surgery improved GU in jejunum (to 20.1 ± 10.6 $\mu\text{mol}/\text{kg}/\text{min}$; $p < 0.005$). Postoperative GU in duodenum (16.9 ± 9.0 ; $p = 0.7$) and colon (16.3 ± 10.3 , NS) remained unchanged. Compared to the healthy group, diabetic and nondiabetic subjects had lower whole body glucose uptake before the operation (12.5 ± 5.3 and 13.3 ± 7.4 $\mu\text{mol}/\text{kg}/\text{min}$ vs.

40,3±9,5 $\mu\text{mol/kg/min}$, $p < 0,001$). Surgery improved whole body insulin sensitivity in both diabetic (to 23,7±8,7 $\mu\text{mol/kg/min}$, $p < 0,01$) and nondiabetic (to 22,1±8,5 $\mu\text{mol/kg/min}$, $p < 0,01$) subjects. Jejunal GU correlated with whole-body insulin sensitivity in all subjects preoperatively ($r = 0,64$, $p < 0,001$) and in the obese postoperatively ($r = 0,75$, $p < 0,001$). Increased postoperative skeletal muscle glucose uptake was associated with improved jejunal ($r = 0,56$, $p < 0,05$) and duodenal GU ($r = 0,68$, $p < 0,01$).

Conclusion: This study demonstrates that obese subjects with and without type 2 diabetes have significant insulin resistance in the proximal intestine. Jejunal GU is improved in line with whole-body and skeletal muscle insulin sensitivity after bariatric surgery and the recovery of diabetes. The findings suggest that intestinal insulin resistance is an important factor in DM2.

Jejunal GU correlates with whole body insulin sensitivity



Clinical Trial Registration Number: NCT00793143

Supported by: EU FB6 (18734, Hepadip), Academy of Finland grant (CoE), Si-grid Juselius

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Endobarrier™ duodenal-jejunal bypass liner rapidly improves diabetes parameters paralleled by increased postprandial GLP-1 and PYY levels in obese type 2 diabetic patients

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Background and aims: Excluding the proximal intestine from nutrients by malabsorptive bariatric surgical techniques improves Type 2 Diabetes (T2DM) within days. The gut peptides Glucagon-like peptide-1 (GLP-1) and Peptide YY (PYY) are thought to play a central role in this important improvement. Here, the effects of the EndoBarrier™ Duodenal-Jejunal Bypass Liner (DJBL), a new minimally invasive duodenal/jejunal bypass sleeve, on diabetes parameters and GLP-1 and PYY were investigated.

Materials and methods: 17 obese T2DM patients received the DJBL in combination with a low calorie diet for 24 weeks. Patients were studied prior to and one week after implantation, and prior to and 1 week after explantation. Blood was sampled before and 10, 20, 30, 60, 90 and 120 minutes after a liquid 500 kcal test meal. HbA_{1c}, glucose, insulin, GLP-1, and PYY concentrations were measured.

Results: At explantation, after 24 weeks, patients showed a mean loss of excess weight of 29.8±3.5% (mean±SEM) while HbA_{1c} had improved significantly from 8.4±0.2% to 7.0±0.2% ($p < 0.01$). Furthermore, anti-diabetic medication was lowered in most patients (16/17). Interestingly, within one week after implantation, fasting and AUC glucose concentrations were improved (11.4±0.5 mmol/L vs. 8.9±0.4 mmol/L and 1,999±88 vs. 1,535±53, both $p < 0.01$). In parallel, AUC PYY and AUC GLP-1 concentrations both increased (2,584±144 vs. 4,112±441 and 4,440±242 vs. 6,448±527, both $p < 0.01$). Both at the time of explantation, and one week thereafter, the glucose parameters, fasting and AUC glucose concentrations, remained significantly decreased while the concentrations of PYY and GLP-1 had returned to normal.

Conclusion: DJBL treatment resulted in significant weight reduction and rapid and long lasting improvement of T2DM. The observed changes in gut peptides shortly after implantation may be involved in the early improvement. These observations are in line with the so-called hindgut hypothesis, which attributes diabetic improvement, after exclusion of the proximal small

intestine, to increased secretion of gut peptides in reaction to presence of undigested nutrients in the distal gut. Other, as yet unrevealed, factors may underlie the sustained effect.

Clinical Trial Registration Number: NCT00985114

Supported by: GI Dynamics

OP 32 Novel agents for type 2 diabetes

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A randomised, double-blind, placebo- and active-controlled, dose-ranging study to determine the efficacy and safety of the novel GPR40 agonist TAK-875 in subjects with type 2 diabetes mellitus

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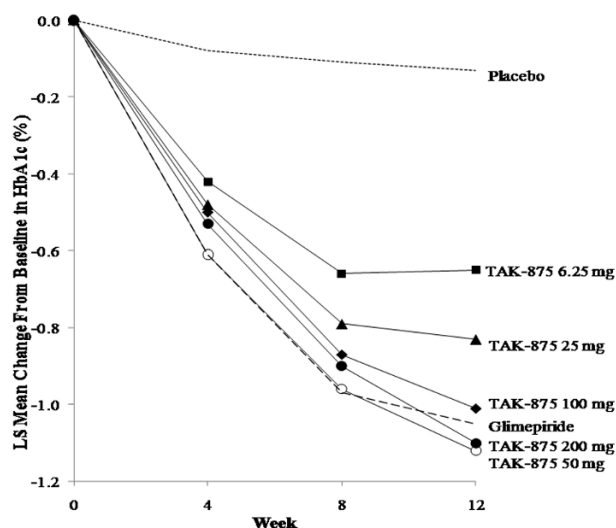
Background and aims: TAK-875 is a selective GPR40 agonist and first in its class to reach clinical development for the treatment of type 2 diabetes mellitus (T2DM).

Materials and methods: A randomized, double-blind, placebo- and active (glimepiride) comparator-controlled, parallel-group, multicenter study was conducted to evaluate the efficacy, safety and tolerability of 5 dosages (ranging from 6.25 mg to 200 mg QD) of TAK-875 over 12 weeks in subjects with T2DM. The primary efficacy endpoint was change from baseline in A1C at week 12. Other endpoints included changes in A1C over time, FPG, plasma glucose post-OGTT, number of subjects rescued due to hyperglycemia and incidence of hypoglycemia.

Results: 426 subjects were randomized (~60 per group). All doses of TAK-875 showed significantly greater A1C reductions at week 12 than placebo (Figure 1). The magnitude of the decrease in A1C produced by TAK-875 ≥50mg was comparable to that with glimepiride at week 12. Compared to placebo, approximately twice as many subjects (33–48%) treated with TAK-875 ≥25 mg achieved HbA1c <7% at week 12, similar to the glimepiride response. Changes from baseline in FPG and 2-hr OGTT values observed with TAK-875 were consistent with changes in HbA1c. The incidence of hypoglycemia was significantly lower in all TAK-875 groups (2.3% of subjects) compared to glimepiride (16.1%) and similar to placebo (3.3%). Treatment-emergent AEs ranged from 43.5% to 61.3% and were highest with glimepiride. Discontinuations because of AEs were low (1.6–3.3%) and similar among all active treatment groups.

Conclusion: The study results provide evidence of good safety, tolerability and HbA1c lowering activity for TAK-875 and are consistent with its glucose dependent mechanism of action. They support further evaluation of this novel compound in the treatment of T2DM.

Figure. Change From Baseline in HbA1c (%) (LOCF) by Study Visit



Clinical Trial Registration Number: NCT01007097

Supported by: Takeda Global Research & Development Center, Inc.

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Orally administered GPR119 agonist PSN821 shows clinically significant glucose lowering and other potential cardiometabolic benefits in patients with type 2 diabetes

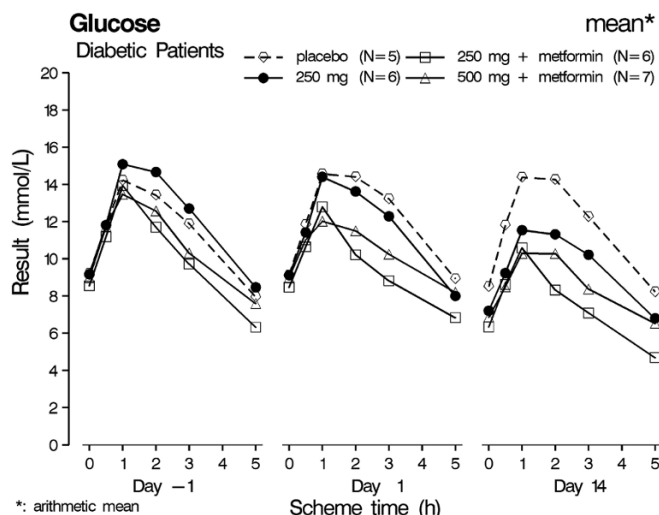
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Background and aims: PSN821 is a potent, selective, orally-administered agonist of the GPR119 receptor expressed predominantly in the pancreas (β-cells) and GI tract (enteroendocrine cells) in humans. In preclinical animal disease models PSN821 has been shown to substantially lower blood glucose and also reduce body weight, potentially via modulation of the gut hormones GLP-1, GIP and PYY. This randomized, double-blind, placebo-controlled assessment evaluated the safety, tolerability, and pharmacodynamic effects of PSN821 in overweight/obese patients with T2DM.

Materials and methods: Three cohorts of patients (250mg PSN821 alone, or 250mg, 500mg PSN821 plus stable metformin therapy) were evaluated in addition to placebo controls. Pharmacodynamic response was assessed by measuring changes in plasma glucose both fasting (C_{pre}) and during a liquid meal (Ensure Plus) challenge (E_{max} and $RAUC_{0-5}$). In addition energy intake at test meal (EI), gut hormones (GLP-1, GIP, PYY), fasting lipid profiles, adiponectin, leptin and weight change were measured.

Results: After 14 days treatment with PSN821 fasting plasma glucose was lower in all actively treated groups (-2.0, -2.1, -2.3 mmol) compared to placebo. Following a liquid meal challenge glucose exposures were lower for all active treatments compared to both baseline and placebo:



Energy intake at test meal was substantially less than placebo at the higher dose (mean -6%, -8%, -40% respectively). Gut hormone profiles were highly variable: GLP-1 and GIP showed no marked difference from placebo after 14 days treatment, however PYY_{total} and PYY₃₋₃₆ appeared increased from day -1, both at Day 14 and compared to placebo at Day 1 and Day 14. For all PSN821 treatments total cholesterol, LDL and triglycerides appeared lower on Day 14 than Day -1, with mean changes being greater than placebo; no differences were observed for HDL in any group. Unequivocal decreases in serum leptin levels (mean -13%, -27%, -15% respectively) and increases in adiponectin (mean +13%, +5%, +18% respectively) were seen in all active treatment groups compared to placebo. Non-statistically significant changes in weight compared to placebo (-1.1, -0.4, -0.7kg respectively) were noted. PSN821 at all doses was well tolerated with a low incidence of gastrointestinal upset and without any adverse effects on labs, vital signs or ECG. There were no SAEs or discontinuations due to AEs.

Conclusion: In this small number of overweight/obese patients with T2DM, PSN821 was well tolerated and showed clear evidence of both fasting and post-prandial glucose lowering, either alone or in combination with metformin. The suppression of EI at higher doses suggests weight lowering potential and corresponding changes in PYY indicate a possible mechanism for this.

Clinical Trial Registration Number: 2009-00927-13

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LY2599506, a novel glucokinase activator (GKA), improves fasting and postprandial glucose in patients with type 2 diabetes mellitusJ.M. Bue-Valleskey¹, K.B. Schneck¹, V.P. Sinha¹, E.T. Wondmagegnehu¹, C. Kapitza², J.W. Miller²;¹Eli Lilly and Company, Indianapolis, USA, ²Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany.

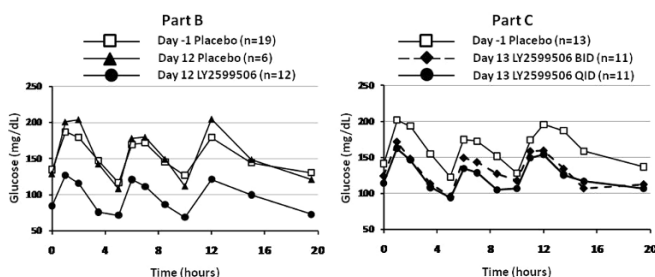
Background and aims: LY2599506 (licensed from Prosidion) is an orally-administered GKA. GKAs lower the plasma glucose threshold for insulin release and may modulate other hormonal and hepatic mechanisms of glucose homeostasis. The primary objective of this Phase 1 study was to assess the safety and tolerability of LY2599506 during multiple-dose administration to healthy subjects and T2DM. Based on previous clinical data, LY2599506 was expected to enhance glucose control for 1–4 hours after dosing; consequently, LY2599506 was given prior to each meal and at bedtime (four times a day [QID] dosing) to achieve glycemic control in both postprandial (PP) and post absorptive periods.

Materials and methods: This 3-part study used a single-blind design with randomized assignment to placebo or LY2599506 (Parts A and B) or study sequence (Part C). In Part A, healthy subjects (n=9) received ~50 mg LY2599506 or placebo QID for 7 days. In Part B, T2DM (n=18) underwent dose-titration of LY2599506 or placebo QID for 12 days; in Part C, T2DM (n=11) received twice-daily (BID) and QID LY2599506 dosing for 13 days each in a 2-period crossover design. Planned LY2599506 dose levels were 50, 100, 200, and 300 mg QID. Starting with 50 mg or 100 mg, doses were increased at 3 day intervals so that blood glucose (BG) thresholds of 60 mg/dL (Part B) and 80 mg/dL (Part C) were not exceeded. Standardized meals at breakfast, lunch, dinner, and bedtime were administered to characterize blood glucose, insulin, and glucagon responses.

Results: At baseline, mean HbA_{1c} in T2DM was 7.4%; mean diabetes duration was 7 years; and 80% were on stable metformin therapy. Mild or moderate hypoglycemia was the dose-limiting adverse event and was effectively managed with food/drink. No severe hypoglycemia occurred, even with dosing at bedtime. QID dosing of LY2599506 achieved a relatively flat 24-h exposure profile. Under inpatient conditions with dose reduction for BG <60 mg/dL (Part B), the maximum tolerated (non-hypoglycemic) dose of LY2599506 ranged from 50–1100 mg/day; under ambulatory conditions with dose reduction for BG <80 mg/dL (Part C), the maximum dose ranged from 60–530 mg/day. In part B, fasting BG after 12 days of treatment was significantly lower during treatment with LY (Placebo: 129 ± 26.6 mg/dL vs LY: 84.4 ± 15.7 mg/dL, p=0.0004) and significant reductions in 2-h PP BG were also observed after each of the main meals (Figure). In Part C, BID dosing was slightly less effective in controlling BG following lunch than QID dosing (Figure). Although absolute differences in PP insulin levels were not generally observed, the insulin/glucose ratios on Day 12 were increased at PP time points relative to placebo (Placebo: 0.36 ± 0.38 vs LY: 0.71 ± 0.33) which may suggest an improvement in beta cell function. No significant effects on glucagon were observed.

Conclusion: LY2599506 was well tolerated and improved glycemic control in T2DM patients.

Mean Glucose Profiles From Test Meal Assessments
Performed on Days -1 and 12 (Part B) or Days -1 and 13 (Part C)



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Short-term treatment with glucagon receptor antagonist LY2409021 effectively reduces fasting blood glucose (FBG) and HbA_{1c} in patients with type 2 diabetes mellitus

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Background: Glucagon levels are elevated in many patients with type 2 diabetes mellitus (T2DM) and contribute to hyperglycemia. LY2409021 is a potent, selective glucagon receptor antagonist.

Materials and methods: This Phase 1, randomized, double-blind, placebo (PBO)-controlled study examined the safety, tolerability, pharmacokinetics (PK), and short-term (28-day) efficacy of once-daily doses of LY (5, 30, 60, or 90mg) in patients with T2DM treated with diet and exercise or metformin (N=47; mean FBG 148 mg/dL; HbA_{1c} 8.0%).

Results: Across LY dose levels, PK parameters t_{max} , $t_{1/2}$, and apparent clearance (CL_{ss}/F) ranged from 6 to 8 h, 56.1 to 61.9 h, and 0.263 to 0.345 L/h, respectively, and C_{max} increased in proportion to dose. Accumulation (R_a) ranged from 3.74 to 4.5. LY produced clinically significant reductions in FBG by Day 2; by Day 14, least squares (LS) mean changes from baseline vs. PBO were -25.74, -39.96, and -37.44 mg/dL (p < .01 for all) in the 30, 60, and 90mg dose groups, respectively. By Day 28, LS mean changes in HbA_{1c} were statistically significant vs. baseline in all treatment groups, including PBO (-0.49%; 90% confidence interval: -0.70 to -0.28), such that significant reductions vs. PBO were seen only at 60- and 90-mg LY, respectively: -0.53% (p=.0117); -0.43% (p=.0391). ED₅₀ for glucose-lowering was 5mg. Fasting glucagon significantly increased by 0.6-, 1.5-, 2.5-, and 4.2-fold vs. baseline across LY dose levels, and fasting active glucagon-like peptide-1 (GLP-1) by 59% at 90mg. Glucagon and GLP-1 returned to baseline levels during follow-up. No significant effects of LY on fasting or postprandial insulin and C-peptide were seen. LY was generally well tolerated. Hypoglycemia was infrequent and mild to moderate in intensity (4 events at 90mg; minimum glucose 62mg/dL). Reversible elevations in hepatic transaminases > 3x ULN were seen in 5 of 9 patients in the 90mg dose group, with no clinical signs or significant elevations in bilirubin or alkaline phosphatase.

Conclusion: The potent glucose-lowering observed during short-term treatment with LY supports the continued development of this glucagon receptor antagonist as a once-daily treatment for T2DM.

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Efficacy and safety of the glucagon receptor antagonist, MK-0893, in combination with metformin or sitagliptin in patients with type 2 diabetes mellitus

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Background and aims: Reduction in hepatic glucose production (HGP) is a key therapeutic goal in patients (pts) with type 2 diabetes (T2DM). Metformin (MET) lowers glucose, in part, by reducing HGP. Sitagliptin (SITA), by suppressing glucagon and enhancing insulin secretion, may also reduce HGP. Inhibition of glucagon action through blockade of the glucagon receptor may provide a complementary mechanism for further reduction in HGP and improvement in glycaemic control. MK-0893 is an oral, highly-selective glucagon receptor antagonist in development for the treatment of T2DM. The present study evaluated the efficacy and safety of combination therapy of MK-0893 with MET or SITA.

Methods: Pts with T2DM were eligible if, at screening, they were either not on an antihyperglycaemic agent (AHA) with an HbA_{1c} of 7.5–12%, or were on AHA monotherapy or low-dose combination therapy with an HbA_{1c} of 7–10%. After AHA wash-off and placebo run-in periods, eligible pts were randomised in a 1:1:1 ratio to double-blind treatment with MK-0893 40 mg qd + MET 1000 mg bid, MK-0893 40 mg qd + SITA 100 mg qd, or SITA 100 mg qd + MET 1000 mg bid. Change in 24-hr weighted mean glucose (WMG) from baseline after 4 weeks was the primary endpoint. Statistical comparisons focused on each of the MK-0893 groups relative to the SITA + MET group.

Results: At baseline, randomised pts (N=146, 61% males) had a mean age of 53 yrs, mean HbA_{1c} of 8.6%, and median duration of T2DM of 7 yrs. All treatment regimens provided substantial reduction in 24-hr WMG and fasting (FPG) and postprandial glucose at Week 4. Therapy with MK-0893 + MET demonstrated statistical superiority to SITA + MET in lowering 24-hr WMG and FPG over 4 weeks (p<0.001), while MK-0893 + SITA was significantly

less effective than SITA + MET (Table). No significant between-group differences were observed for fasting insulin, fasting C-peptide, or HOMA- β . HOMA-IR was reduced to a greater extent with MK-0893 + MET compared with SITA + MET. All treatments were generally well tolerated. A numerically higher incidence of adverse events of diarrhoea was observed in the MK-0893 + MET (10.2%) and SITA + MET (10.2%) groups compared with the MK-0893 + SITA group (0%). Mean changes in ALT levels with MK-0893 + MET, MK-0893 + SITA and SITA + MET were 4.3, -0.4, and -3.2 IU/ml, respectively; mean changes in AST levels were 1.51, 3.85, and -1.59, respectively. Total cholesterol and LDL-cholesterol were increased from baseline with MK-0893 + SITA (mean % change = 3.2% and 4.5%, respectively) relative to reductions with MK-0893 + MET (-0.6% and -5.7%, respectively) and SITA + MET (-5.5% and -7.7%, respectively).

Conclusion: Initial combination therapy of MK-0893 with MET or SITA provided substantial improvements in glycaemic control after 4 weeks. Treatment with MK-0893 in combination with MET or SITA was generally well tolerated. Relative to SITA + MET, small mean increases in hepatic enzymes and plasma lipids were observed with MK-0893 in combination with MET or SITA.

	MK-0893 40 mg qd + MET 1000 mg bid	MK-0893 40 mg qd + SITA 100 mg qd	SITA 100 mg qd + MET 1000 mg bid
Baseline 24-hr WMG, mmol/L	13.19 (3.07)	12.55 (3.27)	12.90 (3.36)
Δ 24-hr WMG, mmol/L	-6.52 (-7.02, -6.02)*	-4.76 (-5.26, -4.25)**	-5.53 (-6.03, -5.02)
Baseline FPG, mmol/L	11.98 (2.88)	11.31 (2.97)	11.46 (2.90)
Δ FPG, mmol/L	-5.65 (-6.12, -5.19)*	-4.09 (-4.57, -3.61)**	-4.60 (-5.07, -4.12)

* $p < 0.001$ or ** $p < 0.05$ vs. SITA 100 mg qd + MET 1000 mg bid.

Baseline data are means (SD) and Δ results are LS mean change from baseline (95% CI)

Clinical Trial Registration Number: clinicaltrials.gov NCT00631488

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Safety and efficacy of oral chemokine receptor 2 antagonist CCX140-B in a phase 2 type 2 diabetes study

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Background and aims: Monocyte chemoattractant protein-1 (MCP-1; also known as chemokine ligand 2, CCL2) and its chemokine receptor CCR2 are implicated in the development of insulin resistance in type 2 diabetes mellitus. CCX140-B is an oral, specific CCR2 antagonist shown previously to be safe and well tolerated in Phase 1 clinical trials. CCR2 antagonist treatment in diet-induced obese mice as well as *db/db* mice has shown significant improvement of glycemic parameters as early as 1 week of treatment.

Materials and methods: A 159-subject multinational, randomized, double-blind, placebo- and active-controlled Phase 2 clinical trial has been completed recently. Subjects had been on stable metformin treatment for at least 8 weeks prior to study entry, had HbA_{1c} of 6.5 to 10% and fasting plasma glucose (FPG) of 135 to 270 mg/dL. Randomized subjects received double-blind placebo QD (N=32), 5mg CCX140-B QD (N=32), 10mg CCX140-B QD (N=63), or open-label pioglitazone 30mg QD (N=32) for 4 weeks. Safety and tolerability were primary endpoints, and secondary endpoints included changes from baseline in glycemic parameters. The pharmacokinetic profile of CCX140 was also evaluated.

Results: Baseline characteristics, mean (SD), were: Age 59 (7) yrs, 64% male, BMI 32 (4) kg/m², median diabetes duration 5.8 yrs, HbA_{1c} 7.5 (0.8) %, FPG 170 (41) mg/dL. CCX140-B was well tolerated by study subjects. No serious adverse events were observed in the CCX140-B groups. There were no safety concerns regarding laboratory hematology, chemistry, or urinalysis. FPG showed a CCX140-B dose-dependent decrease through week 4. HbA_{1c} least-squares mean changes from baseline to week 4 for the placebo, 5 mg CCX140-B, 10 mg CCX140-B, and pioglitazone groups were -0.09%, -0.09%, -0.23% ($p=0.045$ vs. placebo), and -0.13% (NS vs. placebo), respectively. The percentage of subjects showing a decrease from baseline to Week 4 in HbA_{1c} was 58%, 51%, 82% ($p=0.045$ vs. placebo) and 77% (NS vs. placebo) for the placebo, 5 mg, 10 mg CCX140-B, and pioglitazone groups, respectively. Unlike other

CCR2 antagonists, no significant changes were seen in plasma MCP-1 or blood monocyte count with CCX140-B treatment. No significant changes in body weight or evidence of hemodilution or peripheral edema were observed with CCX140-B treatment. At steady state, measured at Day 22, mean (SD) trough plasma levels of CCX140 were 1370 (548) and 2450 (1050) ng/mL for the 5mg and 10mg CCX140-B groups, respectively.

Conclusion: A statistically significant decrease in HbA_{1c} and a higher percentage of HbA_{1c} responders were observed after only 4 weeks of treatment with CCX140-B 10mg QD compared to placebo. No detrimental effects were observed on plasma MCP-1 or blood monocyte levels, and once daily oral CCX140-B provided excellent blockade of blood leukocyte CCR2. The novel oral CCR2-specific antagonist CCX140-B was effective, safe and well tolerated in this study.

Clinical Trial Registration Number: NCT01028963

OP 33 Immunology

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Characterisation of microRNA in sera as biomarkers for type 1 diabetes progression in newly diagnosed children

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Background and aims: The use of serum/plasma samples as a non-invasive and easily accessible body fluid for detection of the miRNAs has shown to be of great potential in the clinic as a reliable way to detect cellular and physiological changes as cancer, pregnancy and sepsis. This study aim to identify specific key miRNAs and miRNA patterns usable as clinical biomarkers, which can be predictive for ongoing beta-cell function destruction and regeneration in children with newly diagnosed type 1 diabetes (T1D). Therefore, we have investigated miRNAs in fractionated serum samples from two cohorts of new-onset T1D children and age-matched controls by miRNA sequencing and validation by qRT-PCR.

Materials and methods: The International Hvidoere Cohort and the Danish Remission Cohort comprise 275 and 130 newly diagnosed children (0.2–16.8 years), respectively. The residual beta-cell function was estimated by a meal-stimulated C-peptide test 1, (3 for the Danish Cohort), 6 and 12 months after disease onset and blood samples for centrally determined HbA_{1c}, stimulated C-peptide, glucagon, incretin hormones, cytokines and immunology was taken. DNA was extracted for SNPs genotyping. In addition 151 healthy children (6.7–13.7 years), which were recruited from 2 public schools in the Copenhagen area, served as a control group. Pooled RNA samples (containing 5–10 µg RNA) from sera collected 1 month after diagnosis were sequenced on the Solexa sequencing platform (Illumina). The deep-sequencing data were analysed with bioinformatics and statistical tools and differentially expressed miRNAs were validated by qRT-PCR.

Results: By using Solexa sequencing 1,100 miRNAs were found to be expressed in serum samples from the three cohorts. 47 miRNAs met the criteria of copy number >100 and a 2-fold altered expression in both diabetes cohorts. Of these 26 miRNAs were selected for further validation by RT-qPCR in the individual samples. Among the 26 miRNAs we found several miRNAs already known to be linked to insulin production and glucose responsiveness (miR-124 and miR-200), apoptosis (miR-21) and beta-cell regulatory networks (miR-192 and miR-199a). Even more importantly we found several miRNAs with yet unidentified function related to T1D (e.g. miR-24, miR-25 and miR220).

Conclusion: This study demonstrates the feasibility of identifying miRNAs in serum that associate with newly diagnosed T1D. Further studies of the key miRNAs may help identify new pathways involved in the pathogenesis of beta-cell destruction in T1D.

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Incidence of multiple autoimmune phenotypes among children at high risk for type 1 diabetes

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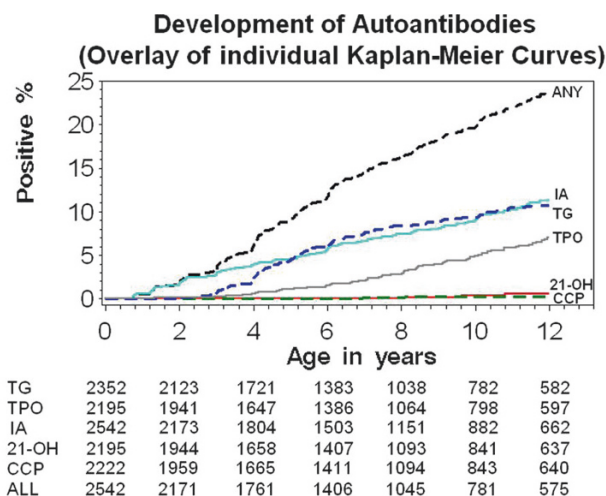
Background and aims: HLA-DR3,DQ2 and DR4,DQ8 haplotypes are associated with multiple autoimmune diseases. However, little is known concerning the overall autoimmunity burden among children with these HLA markers.

Materials and methods: The Diabetes Autoimmunity Study in the Young (DAISY) has followed for an average of 8 years 2542 children at risk for T1D, recruited by newborn screening for HLA DR3,DQ2 or DR4,DQ8, or as relatives of T1D patients. Participants were tested annually for autoantibodies to glutamic acid decarboxylase 65 (GAD), insulin (IAA), insulinoma associated antigen-2 (IA2) and transglutaminase (TG). The most recent sample was measured for zinc transporter 8 (ZnT8), thyroid peroxidase (TPO), 21-hydroxylase (21-OH) and cyclic citrullinated peptide (CCP); if positive, the entire subject sample series was tested. GAD, IAA, IA2 and ZnT8 are grouped as

islet autoantibodies (IA). Positivity on 2 or more consecutive tests defined the specific autoimmune phenotype. Standard clinical criteria defined autoimmune disease.

Results: By the age of 12 years, one in four children expressed an autoimmune phenotype: 23.8% (95% CI: 21.5–26.1) and 12.9% had more than one autoimmune phenotype. IA developed in 11.3% (9.6–12.9), TG in 10.8% (9.1–12.4), TPO in 7.0% (5.4–8.6), 21-OH in 0.5% (0.1–1), and CCP in 0.2% (0.0–0.5). Overall 5% of the children have developed at least one clinically confirmed disease: 69 T1D, 52 celiac disease, 9 hypothyroidism, 1 Addison's disease, and 2 rheumatoid arthritis. The HLA genotypes most strongly associated with autoimmune phenotypes were: DR3/4,DQ8 for T1D Relative Risk =2.8 (2.0–3.9), DR3/3 for TG 2.4 (1.5–3.8), and DR4/4,DQ8 for TPO 2.0 (1.1–3.5), adjusting for sex, ethnicity, and family history of T1D.

Conclusion: The high incidence of autoimmune phenotypes in children at genetic risk for T1D warrants a screening program and early intervention.



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Optimising the tolerogenic potential of a 'vaccine' for type 1 diabetes by topical betamethasone therapy

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Background: Peptide immunotherapy has been shown to be a simple and effective method of restoring tolerance and reversing disease in animal models of type 1 diabetes. In humans, the intradermal route of peptide administration is convenient. It is highly important to administer the peptide into an environment in which antigen presenting cells such as dendritic cells are maintained in a tolerogenic state to maximize their potential of inducing tolerogenic responses. Use of the intradermal route has a specific advantage in peptide immunotherapy as dendritic cells are potentially accessible for peptide immunotherapy and local treatment with agents may be able to promote the induction of tolerance.

Aims: Our aim was to determine whether topical pre-treatment of the skin has the potential to promote a tolerogenic phenotype in epidermal dendritic cells.

Materials and methods: Healthy volunteers received no treatment (C) or one of 5 pre-treatment topical regimes twice/daily for 4 days followed by a 50 µl intradermal saline injection: no treatment (Sal), 0.05% Betamethasone (S), 50 mcg/mg Calcipotriol (D), 0.05% Betamethasone + Calcipotriol (S+D), 0.01% Tretinoin (A). 12–18 hours later, all subjects had a 10–15 mm suction blister raised at the site of treatment. Blister fluid was then withdrawn for the measurement of cytokine production. The blister roof was removed and epidermal cells isolated for study using 9-colour flow cytometry and for functional analysis in a Mixed Lymphocyte Reaction (MLR) using allogeneic PBMC.

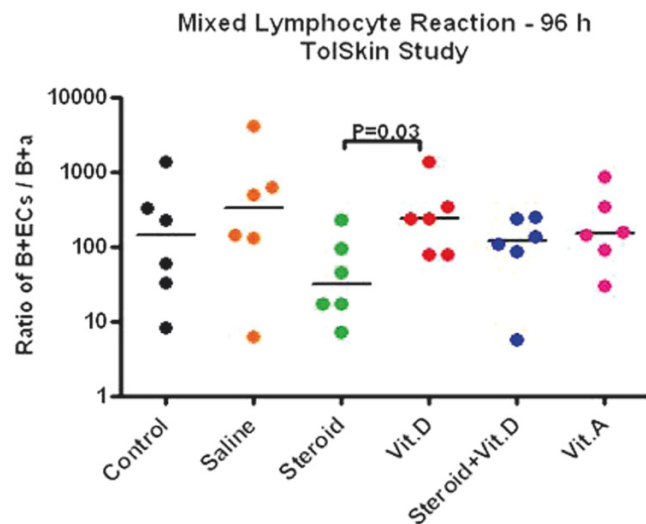
Results: Epidermal cells comprised around 2% CD1a+ dendritic cells (EDC), which were 80–90% viable and induced a strong MLR response in control subjects (Sal).

DCs from S treated subjects showed a trend to reduced MLR proliferation less than Calcipotriol (P=0.03), and lower levels of TNF-α and MCP-1 in the

interstitial (blister) fluid in comparison with Calcipotriol Group ($P=0.01$, $P=0.002$ respectively). Levels of TNF- α and MCP-1 in the Steroid group were significantly lower than the levels in the saline group ($P=0.05$, $P=0.05$ respectively). There was also significant reduction in the levels of expression of the key costimulatory molecules, CD86 and CD83 with the Betamethasone topical therapy in comparison with the saline group ($P=0.04$, $P=0.04$ respectively). A trend to lower levels of expression of CD40, CD80 and ILT3 was seen with vitamin D. No significant changes were observed with vitamin A pretreatment in this dataset.

Conclusion: Pretreatment of the skin with steroid (Betamethasone) for 4 days appears to have significant effects on epidermal dendritic cell phenotype and cytokine milieu. Further functional studies are required to determine whether these changes will prove beneficial in tolerance induction in peptide immunotherapy.

41% in the last quartile, $p=0.013$) after adjustment for age and genetic risk. **Conclusion:** The rising incidence of type 1 diabetes in the UK between 1985–2002 was paralleled by changes in islet autoantibodies at diagnosis indicating broader reactivity against target epitopes, independent of changes in age at diagnosis and HLA class II genetic risk. This suggests that changes in the disease process may underlie recent increases in childhood diabetes. *Supported by: Diabetes UK*



Clinical Trial Registration Number: CT646

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Increases in number, prevalence and level of islet autoantibodies at diagnosis of childhood type 1 diabetes parallel the rising incidence: evidence for a changing disease process?

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Background and aims: The incidence of type 1 diabetes is increasing, and islet autoantibodies are important markers of immune activity. Our aim was to determine whether autoantibodies at diagnosis altered during a period of rapid increase in diabetes incidence.

Materials and methods: We studied 611 patients from the Oxford Region, UK with type 1 diabetes diagnosed before age 21 years (median 11.0 years) between 1985 and 2002. Sera were collected within 3 months of diagnosis (median 1 day). Autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA), islet antigen-2 (IA-2A), the juxta-membrane domain of IA-2 (JMA), the PTP region of IA-2 β (IA-2 β A) and zinc transporter 8 (ZnT8A) were determined by radioimmunoassay. Patients were divided into four groups on the basis of quartiles of date of diagnosis (Jul 1985–March 1990, April 1990–February 1994, February 1994–June 1997, and July 1997–February 2002). Logistic regression was used to determine whether autoantibody prevalence varied according to date of diagnosis after adjustment for age, gender and HLA class II genetic risk.

Results: The prevalence of IA-2A increased from 69% to 83% between the first and last date of diagnosis quartile (logistic regression, $p=0.025$), and prevalence of ZnT8A from 64% to 75% ($p=0.022$). This was mirrored by increased levels of IA-2A ($p=0.025$), ZnT8A ($p<0.01$). Amongst IA-2A positive patients, IA-2 β A levels also increased ($p<0.001$). IAA, GADA and JMA did not change with respect to date of diagnosis. The number of autoantibodies detected (in 422 patient samples collected within 2 weeks of diagnosis, based on IAA, GADA, IA-2A and ZnT8A) also increased ($p=0.02$), and patients in the more recent group were more likely to have all four autoantibodies (27% in the first quartile and

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Mesenchymal stem-cells derived microvesicles modulate cellular immune response to islet antigen GAD in type 1 diabetes

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Background and aims: Mesenchymal stem cells (MSCs) exert an immunosuppressive effect on immune system and can abrogate *in vitro* the pro-inflammatory Th1 response to islet antigen GAD in type 1 diabetes by impairing the production of IFN- γ . The mechanism may involve paracrine factors. Microvesicles (MVs) released from MSCs may account for this paracrine mechanism through a horizontal transfer of messenger RNA and microRNA. In the present study we evaluated whether MSC-derived MVs exert immunomodulatory effect similar to that of MSCs on T cell responses against GAD in type 1 diabetes.

Materials and methods: Human MSCs were isolated and characterised and MVs were purified from supernatants of MSCs by differential ultracentrifugation. The size of MVs was determined by the Zetasizer Nano instrument and by electron microscopy. Moreover, on MVs, cytofluorimetric analyses was assessed to analyse adhesion molecules and gene array analyses was performed to detect their mRNA content (Illumina platform) and miRNA profile was evaluated by quantitative real time PCR (microRNA human panel early accesskit). Peripheral blood mononuclear cell (PBMCs) were obtained from 4 type 1 diabetic patients at disease onset with a positive IFN- γ response to GAD65 stimulation by ELISPOT assay (positive response SI \geq 3). PBMCs were pulsed with GAD65 and incubated with or without MV for 24h followed by IFN- γ ELISPOT. Levels of prostaglandin E $_2$ (PGE $_2$), TGF- β , IL-10, IFN- γ , IL-6, in supernatants were measured by ELISA.

Results: By cytofluorimetric analyses MVs MSC-derived were detected mainly below the forward scatter signal corresponding to 1- μ m beads. When determined by Zetasizer, the size of MVs was ranging from 80 nm to 1 μ m, with a mean value of 135 nm. Transmission as well as scanning electron microscopy performed on purified MVs showed their spheroid morphology and confirmed their size. Cytofluorimetric analyses showed the presence of several adhesion molecules known to be expressed on MSC plasma membrane such as CD44, CD29, α 4- and α 5- integrins and CD73, but not α 6-integrin. In addition, MVs did not express HLA-class I molecule at variance of the cells of origin. MVs obtained from MSCs contained mRNA and in particular mRNA related to immune regulation, such as Cytokine receptor-like factor 1 (CRLF1), Interleukin 1 receptor antagonist (IL1RN), Metallothionein 1X (MT1X). Incubation of PBMCs obtained from the four GAD-responder diabetic patients with MVs, resulted in a significant decrease in the number of IFN- γ spots (mean spots 28 ± 2.8 without MVs, mean spots 14 ± 6.7). Furthermore, levels of IFN- γ , IL-6, in supernatants of GAD65-pulsed PBMCs incubated with MVs were significantly decreased compare to GAD65-pulsed PBMCs alone. Levels of PGE $_2$, TGF- β and IL-10 were significantly increased compare to GAD65-pulsed PBMCs alone.

Conclusion: These results provide evident that MSC-derived microvesicle inhibited *in vitro* a pro-inflammatory Th1 response to an islet antigenic stimulus, in diabetic patients, possibly mediated by PGE $_2$ and TGF- β . MVs induce IL-10 secretion, suggesting a possible switch to an anti-inflammatory Th2 signalling of T cells.

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GAD65Ab specific anti-idiotypic antibody prevents the onset of type 1 diabetes in NOD mice

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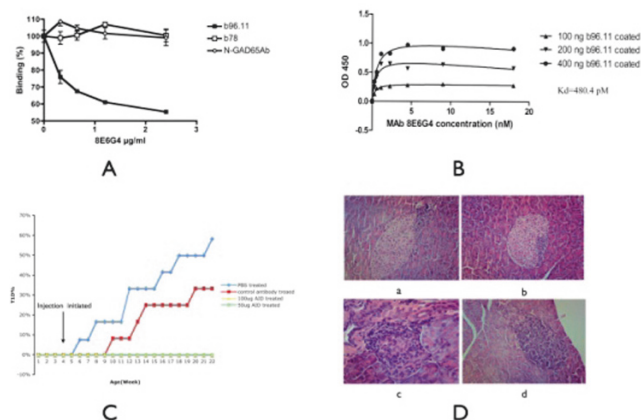
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Background and aims: We previously found anti-idiotypic antibodies (AID) targeting human GAD65 auto-antibody (GAD65Ab) in the healthy population and their significantly decreased levels in patients with type 1 diabetes (T1D). Induction of GAD65Ab targeting AID by injection of human GAD65Ab b96.11 reduced morbidity of T1D in NOD mice. The aim of this study is to develop a monoclonal AID specifically binding to the antigen binding site of human GAD65Ab b96.11 for the prevention of T1D.

Materials and methods: The monoclonal GAD65Ab specific AID was developed by screening the hybridoma cells fused from the B lymphocytes of

Balb/c mice immunized with human GAD65Ab b96. The binding specific of the AID to the GAD65Ab b96.11 was characterized by ELISA, immunoprecipitation, and competing radioligand binding assay (RBA). The binding affinity of the AID to human GAD65Ab b96.11 was calculated by non-competitive ELISA. For *in vivo* study, female NOD mice at the age of 3–4 weeks were intraperitoneally injected with either 50 or 100 μ g of the monoclonal AID weekly. Control groups were injected with either PBS or non-related murine monoclonal antibody. Blood glucose was determined weekly by Abbott Optium Xceed glucometer. The onset of diabetes was defined as two consecutive blood glucose detected higher than 250mg/dL. For histopathological assay, pancreas of mice in each group was isolated and fixed in 4% paraformaldehyde and embedded in paraffin wax. Sections of 4- μ m were mounted on glass slides and stained with haematoxylin and eosin for histological analysis. **Results:** One monoclonal antibody, named 8E6G4, was screened out as the human GAD65Ab b96.11 specific AID. This AID specifically bound to b96.11 and blocked its interaction with GAD65, indicating the AID targets antigen binding site of b96.11. The dissociation constant of the AID to GAD65Ab b96.11 calculated by the non-competitive ELISA is 480.4pM. Our ongoing study in the NOD mice showed this monoclonal GAD65Ab specific AID, but not the control antibody, prevented the onset of T1D after 18-week treatment. Histopathological assay showed the AID reduced insulinitis in NOD mice.

Conclusion: Monoclonal antibody 8E6G4 is a human GAD65Ab b96.11 specific AID which has the potential to prevent T1D by reducing insulinitis.



A: Monoclonal antibody 8E6G4 specifically blocked binding of b96.11 to GAD65 in RBA: Binding of monoclonal antibodies b96.11 (black squares), b78 (white squares), and N-GAD65mAb (white circles) to radiolabeled GAD65 in the presence of the indicated concentrations of 8E6G4 was determined. Binding is reported as percent binding, binding of the respective antibody to radiolabeled GAD65 in the absence of 8E6G4 is set as 100%. We found that only binding of b96.11 to GAD65 was inhibited by 8E6G4, while both b78 and N-GAD65Ab were not affected by 8E6G4. These findings support that 8E6G4 is a b96.11 specific anti-idiotypic antibody, that binds to the antigen-binding region of b96.11; B: Dissociation constant of the AID 8E6G4 to b96.11 calculated by non-competitive ELISA is 480.4pM: binding of serially diluted AID (0.256–16.4nM) to different amount of b96.11 (1, 2, and 4ng/ μ l) coated in 96-well plate was analyzed by standard ELISA. The concentration of 8E6G4 and OD₄₅₀ were plotted to three hyperbolic curves and each K_d was calculated. The K_d was calculated average value of the following formula: $K_d = 2(\alpha K_d' - K_d)/(n-1)$, where $n = [b96.11]/[b96.11]_0$; C: Injection of the GAD65Ab specific monoclonal AID reduced morbidity of T1D in NOD mice in the 18-week intervention: Female NOD mice from 4-week age were weekly injected with 100 μ g GAD65Ab specific AID (yellow triangle), 50 μ g AID (green circle), 100 μ g control AID (red square) or PBS (blue diamond), onset of T1D was monitored by testing blood glucose weekly; D: GAD65Ab specific monoclonal AID reduced the insulinitis in NOD mice: a-d are representative image from mice treated with 100 μ g AID(a), 50 μ g AID(b), 100 μ g control antibody(c) and PBS(d).

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OP 34 Hypoxia and remodelling of adipose tissue

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Differentiation of human adipocytes at different oxygen levels results in increased adiponectin secretion but impaired adipocyte insulin signalling

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Background and aims: Obesity is characterized by a low grade inflammation of adipose tissue (AT) and dysregulation of AT secretory function of enlarged adipocytes. Hypoxia has been demonstrated to occur in AT of obese subjects and hypoxia treatment of adipocytes revealed dysregulation of adipocyte secretion with increase in inflammatory adipokines and reduction of adiponectin release. *In vitro* studies using adipocytes are mostly performed under normoxic conditions with a short-term exposure to strong hypoxia after differentiation. The aim of this study was to differentiate human primary adipocytes under physiological conditions of normal tissue oxygenation and to investigate its effect on adipocyte secretion and insulin signaling.

Materials and methods: Human primary adipocytes were differentiated under 5 %, 10 % and 21 % O₂ in an Xvivo hypoxic chamber. Conditioned medium (CM) and cell lysates were collected and analyzed by ELISA and Western Blotting. Fully differentiated adipocytes were treated with 100 nM insulin and insulin signaling was analyzed at the level of Akt phosphorylation. For analyzing the cross-talk between adipocytes and human skeletal muscle cells (hSkMC), hSkMC were incubated over night with the different CM and insulin signaling was investigated.

Results: Differentiation of adipocytes under 5 or 10 % O₂ resulted in unchanged adipocyte formation as demonstrated by lipid accumulation compared to 21 % O₂. However, analysis of adipocyte release revealed substantial differences in the secretion of several adipokines after differentiation at different O₂ concentrations. Adiponectin secretion was significantly elevated in CM of adipocytes differentiated under 5 and 10 % compared to 21 % O₂ (46.1 vs. 74.3 vs. 25.2 ng/ml), whereas reduced leptin levels under 10 % O₂ could be observed (21.7 vs. 14.7 vs. 27.9 pg/ml). Furthermore, release of the novel adipokine DPP-4 was elevated at 5 and 10 % compared to 21 % O₂ (2.1 vs. 3.2 vs. 1.3 ng/ml), whereas IL-6 amounts were reduced in CM at 5 %, but increased in CM of adipocytes differentiated at 10 % compared to 21 % O₂ (48.6 vs. 389.7 vs. 93.6 pg/ml). Analysis of insulin signaling showed a reduced stimulation of Akt phosphorylation by 30 to 40 % in adipocytes differentiated at 5 and 10 % compared to 21 % O₂. Expression analysis revealed an elevation in the expression level of the insulin receptor (IR)-β in adipocytes differentiated under 5 and 10 % compared to 21 % O₂ (2.3 vs. 1.6 fold). Additionally, incubation of hSkMC with CM from adipocytes differentiated under 5 and 21 % O₂ showed a substantial reduction in insulin-stimulated phosphorylation of Akt that was however comparable between both CM (43 % vs. 45 %).

Conclusion: We demonstrate that reduction in oxygen supply during differentiation of human primary adipocytes mimicking physiological tissue oxygen levels does not impair adipogenesis but leads to alterations in adipokine secretion. Especially the oxygen sensitive factor adiponectin shows a substantial elevated level in CM of adipocytes that were differentiated under more physiological concentrations. Furthermore, we observed impaired insulin signaling of adipocytes differentiated at 5 and 10 % O₂ although the expression of the insulin receptor is elevated. This study demonstrates that adipocyte secretion and signaling is highly oxygen sensitive illustrating hypoxia as an important factor in adipocyte biology that might be important in obesity-induced metabolic diseases.

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Heme oxygenase-1 specifically modulates pro- and anti-adipogenic molecules to inhibit white adipose differentiation

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Background and aims: Adipocyte differentiation is a highly regulated process controlled by a complex signaling network. Mounting evidence suggests an essential role for Heme Oxygenase-1 (HO-1) in adipocyte generation and

function. However, the underlying mechanisms are currently unknown. Here, we aimed to investigate a potential mechanism by which HO-1 regulates adipogenesis.

Material and methods: Construction of transgenic mice was generated with the Cre-loxP system ultimately ablating HO-1 in adipocytes *in vivo*. Transgenic cell lines were made for studying constitutive stable HO-1 knock-down or overexpression in 3T3-L1 preadipocytes. Adipocyte differentiation studies were performed on isolated stromal vascular fractions from transgenic mice (containing preadipocytes) and 3T3-L1 cells.

Results: HO-1 is expressed in 3T3-L1 preadipocytes and declines during initial differentiation. Overexpression of HO-1 in 3T3-L1 cells prevents adipogenesis, whereas shRNA-mediated reduction of HO-1, promotes differentiation, as shown by increased lipid accumulation and elevated expression of adipogenic transcription factors PPARγ, CEBPα, KLF-15 and adipocyte marker genes aP2 and Adiponectin. HO-1 seems specific for white adipose tissue differentiation as knockdown of HO-1 did not affect brown adipocyte (HIB-1B) differentiation. Our findings with 3T3-L1 cells were substantiated with HO-1 deleted preadipocytes isolated from HO-1loxP/loxP mice: Adeno-Cre virus mediated excision of HO-1 *in vitro*, followed by differentiation resulted in enhanced accumulation of neutral lipids, as compared to HO-1loxP/loxP preadipocytes infected with Adeno-GFP virus. GeneChip comparisons of control- and HO-1 overexpressing 3T3-L1 cells induced for differentiation over a time course revealed several genes modulated by HO-1 including transcription factors, signaling proteins, as well as unknown genes. Some of these genes are now being investigated in functional assays as potential mediators of the anti-adipogenic effects of HO-1.

Conclusion: Collectively, our data demonstrate an inhibitory function of HO-1 in (white) adipocyte differentiation via specific modulation of pro-adipogenic and anti-adipogenic molecules.

Supported by: WWTF, FWF, CCHD, MUV

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The canonical Wnt activator, WISP 2, is upregulated in hypertrophic obesity and prevents stem cell commitment and adipocyte differentiation

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Background and aims: The rapidly escalating prevalence of obesity is the major driver of the ongoing global epidemic of type 2 diabetes. Recent evidence indicates that an inability to normally store excess lipids in the subcutaneous adipose tissue leads to ectopic fat accumulation with insulin resistance and the associated dysmetabolic state. This, in turn, is a likely consequence of a reduced ability to recruit and differentiate new adipose cells leading to hypertrophic obesity. We have recently found that individuals with a genetic predisposition for type 2 diabetes, but not for obesity, are prone to develop hypertrophic obesity following small increases in body weight. This suggests that signaling pathways regulating mesenchymal stem cell commitment and/or preadipocyte differentiation are dysregulated in hypertrophic obesity. The canonical Wnt pathway is a key regulator of precursor cell differentiation to adipose cells. We have found that this pathway is upregulated in hypertrophic obesity and that Wnt-inducible secreted protein 2 (WISP 2) is a mediator of this pathway. We here examined the effect of WISP 2 on human mesenchymal stem cell (hMSC) commitment and differentiation and also if fully differentiated adipose cells are target cells for this secreted protein.

Materials and methods: hMSC were cultured with established protocols and induction of adipogenesis was characterized in the presence or absence of WISP 2 or the canonical Wnt ligand Wnt 3a after 12 and 21 days. 3T3-L1 cells were differentiated to adipose cells and then grown under basal conditions or in the presence of WISP 2 or Wnt 3a for 4 days. RNA and protein were harvested and gene expression measured with qPCR.

Results: WISP 2, like Wnt 3a, prevented hMSC commitment and differentiation to adipocytes measured both in terms of lipid accumulation and gene expression of typical adipocyte markers at both time points; C/EBPα (12d, p<0.01; 21d, p<0.01), FABP4 (12d, p<0.01; 21d, p<0.05), Adiponectin (12d, p<0.001; 21d, p=0.07), GLUT4 (12d, p<0.05; 21d, p<0.01). These effects were similar to those seen with Wnt 3a. Thus, these findings are consistent with WISP 2 as a mediator of the canonical Wnt pathway and an inhibitor of stem cell commitment and adipogenesis. We also examined if differentiated adipose cells are targets for Wnt activation by culturing mature 3T3-L1 adipocytes with WISP 2 or Wnt 3a for 4 days. WISP 2 induced a de-differentiation of the adipose cells measured as expression of adipogenic markers including C/EBPα (p<0.05), GLUT4 (p<0.05) and LPL (p<0.05), similar to the effects of Wnt 3a which is in agreement with our previous work. This downregulation of the adipose

cell phenotype impairs the ability of the cells to accumulate and store lipids. **Conclusion:** These results show that WISP 2, like Wnt 3a, inhibits both commitment and differentiation of adipose precursor cells to the mature adipose phenotype which, in turn, also prevents the cells from storing excess lipids and to secrete adipokines which promote insulin sensitivity like adiponectin. Furthermore, fully differentiated adipose cells undergo de-differentiation which also makes these cells targets for the Wnt pathway. Since WISP 2 inhibits PPAR γ and is upregulated in the adipose tissue in hypertrophic obesity, the present findings support this molecule as an important factor behind the metabolic complications of obesity and the Metabolic Syndrome.

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Nutritional reversibility of human adipose tissue hypoxia and fibrosis

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Background and aim of the study: Adipose tissue (AT) of obese subjects is known to be hypoxic, fibrosed and inflamed which contributes to metabolic dysfunction including diabetes. Collagen VI α III and the metalloproteinase MMP14 which is involved in collagen breakdown have previously been associated with the pathogenesis of diabetes. However, little is known about whether hypoxia and the fibrosis-related extracellular matrix changes (ECM) in adipose tissue (AT) are reversible by caloric restriction. The aim of this study was to examine hypoxia and fibrosis markers, collagens and collagenases in abdominal subcutaneous AT (SCAT) of subjects 1) before and after a very-low-calorie diet (VLCD) and subsequent re-feeding and 2) after fast food based hyper-alimentation induced weight gain.

Materials and methods: For the VLCD study, 24 obese subjects (BMI=37.6 \pm 4.9 kg/m²) underwent a 450 kcal/day based diet for 16 weeks (wks) followed by 2 wks of food reintroduction. Six lean subjects (BMI=21.4 \pm 2.5 kg/m²) underwent a fast food based diet for 4 wks. SCAT-biopsies were performed at 0, 8, 16 wks of caloric restriction by VLCD and at 2 wks after re-feeding. In the hyperalimantation group, biopsies were taken at 0 and 8 wks. ECM components and fibrosis regulators (16 collagens, fibronectin, TGF- β and MMPs) were studied alongside the hypoxia marker HIF-1 α using DNA microarray technology.

Results: With VLCD subjects lost 28 \pm 3.7 kg of weight and weight did not change in the period of food reintroduction. The mean weight gain in the hyperalimantation study was 7.2 \pm 1.6 kg. After VLCD induced weight loss there was no change in collagen Ia1 and Ia2 gene expression, however, within 2 wks of re-feeding, collagen expression increased (42%, p <0.01 and 29%, p <0.001 respectively) responded similar to hyper-alimentation in which collagen Ia1 and Ia2 were also up-regulated by 39% (p =0.01) and 48% (p <0.05) respectively. Collagen VI α 3 expression increased after VLCD (18%, p <0.001) but not significantly with hyper-alimentation and did not respond to re-feeding. With VLCD fibronectin decreased (36%, p <0.001), MMP14 increased (19%, p <0.001), and TGF- β and HIF-1 α remained unchanged; and as expected TGF- β , fibronectin and HIF-1 α increased with hyper-alimentation (p <0.01). In the period of food reintroduction only fibronectin decreased further (15%, p <0.001) whilst TGF- β and HIF-1 α and MMP14 remained unchanged.

Conclusion: Diet induced weight loss fails to reduce expression of the fibrosis inducing growth factor TGF- β , collagen VI α III and the hypoxia responsive gene HIF-1 α . The expression of collagen 1, the most abundant collagen in AT, continues to be up-regulated during weight loss and is hyper-responsive to the nutritional challenge of re-feeding with rapid up-regulation even before weight regain occurs. Whilst metalloproteinases may in part balance increased collagen production with increased collagen turnover during weight loss, the period of refeeding may be prone to an imbalance and subsequent dysfunction. Cyclical dieting also known as yo-yo dieting may thus prime SCAT towards fibrosis and to associated unfavourable metabolic consequences.

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Dual effects of sphingosine 1-phosphate (S1P) on proliferation of mature adipocytes

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Background and aims: We have reported that mature adipocytes proliferate regulated with proliferin (PLF) in the last scientific session. 5-Bromo-2'-deoxyuridine (BrdU), a thymidine analog, incorporation, as well as expression of proliferating cell nuclear antigen (PCNA), was observed in mature adipocytes isolated from Wistar rats using collagenase digestion. In addition, isolated mature adipocytes were cultured with ceiling culture method, which revealed the population exhibiting dramatically replicating ability. Furthermore, dividing nuclei and centrosomes were identified with immunocytochemical staining using anti- γ -tubulin antibody. On the other hand, increased cell number, which was assessed with hemocytometer and MTT assay, BrdU incorporation and expression of PCNA were detected in fully differentiated 3T3-L1 adipocytes. Cell cycle was clearly recognized with flow cytometry in mature 3T3-L1 adipocytes. As reported previously, we took note of PLF as a growth factor in adipocytes. Gene silencing with siRNA, as well as adding anti-PLF antibody to the culture medium, suppressed cell proliferation in mature 3T3-L1 adipocytes proliferation. Considering the fact that PLF signaling is sensitive to pertussis toxin, we speculated the involvement of S1P in mature adipocyte proliferation. Recent research identified G protein-coupled five S1P receptors which were named S1P₁-S1P₅. Therefore, we evaluated the effect of S1P and inhibition of S1P₁ and S1P₂ on mature adipocytes proliferation.

Materials and methods: Effects of 1 μ M S1P, 10 μ M FTY-720, S1P₁ agonist, and 10 μ M JTE-013, a specific S1P₂ antagonist, on mature 3T3-L1 adipocytes proliferation were measured with BrdU incorporation. We also assessed the impacts of gene silencing of S1P₁ and S1P₂ with siRNA. Moreover, Effects of intraperitoneal injection with JTE-013 (2mg/kg) or FTY-720 (4mg/kg) on mature adipocytes were examined. Animal care and experimental procedures were performed under the approval of the Animal Care Committees of our University.

Results: Treatment with S1P increased BrdU incorporation, while gene silencing S1P₁ with siRNA reduced it. Inhibiting S1P₂ with JTE-013 or knock-down of S1P₂ with siRNA increased it in mature 3T3-L1 adipocytes. Treatment with FTY-720 increased BrdU incorporation in mature 3T3-L1 adipocytes. Taking together, these results indicate S1P₁ and S1P₂ act oppositely on mature adipocyte proliferation, whereas proliferating effect is predominant. Administration of JTE-013 or FTY-720 increased the expression of PCNA in isolated mature adipocytes from epididymal fat of C57/Black mice. Interestingly, treatment with JTE-013 for 2w resulted in suppressed body weight increase and the tendency to lower plasma glucose level in ob/ob mice. These results suggest that accelerated cell proliferation in mature adipocytes might lead to improvement of obesity and diabetes.

Conclusion: S1P regulates mature adipocytes proliferation, which influences glucose and lipid metabolism in whole body.

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The adipogenic potential of human adipose tissue precursor cells is regulated in a fat depot- and BMI-dependent manner

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Background and aims: An impaired lipo-/adipogenic capacity of human adipose stem cells (hASCs) may contribute to abdominal obesity and the related metabolic abnormalities. The aim of this work was to investigate whether obesity is associated with changes in the adipogenic potential of hASCs from the subcutaneous (SC) and visceral (V) adipose tissues.

Materials and methods: hASCs were isolated from paired SC and V fat biopsies, obtained from 10 lean (BMI 24.1 \pm 2.3 kg/m²) and 10 obese (BMI 37.4 \pm 3.2 kg/m²) subjects. Cells were differentiated in vitro in the presence of insulin and other adipogenic inducers, and adipogenesis was evaluated by analyzing the lipid deposition by Oil-Red-O staining, lipid droplet area by a dedicated image processing software, and gene expression by qRT-PCR.

Results: In lean subjects, the proportion of terminally differentiated adipocytes derived from SC hASC was significantly higher compared to that from V hASC, along with the estimated lipid droplet size, lipid staining intensity, and mRNA expression levels of PPAR γ 2 and GLUT4 ($p<0.05$). When SC hASC from lean and obese subjects were induced to differentiate comparatively, no significant differences in the proportion of mature adipocytes or PPAR γ 2/GLUT4 expression were detected, even though the fraction of adipose cells with large lipid droplets was higher in obese SC compared with lean SC cell cultures ($p<0.05$). By contrast, a markedly greater number of mature adipocytes, along with larger lipid droplets, and higher PPAR γ 2 and GLUT4 mRNA levels were observed in cultures derived from obese V as compared to lean V hASCs ($p<0.05$). Furthermore, insulin (10–100 nM) increased Akt phosphorylation to a greater extent in obese V as compared to lean V hASCs ($p<0.05$), whereas no differences in ERK-1/2 activation were observed.

Conclusion: Therefore, fat precursor cells isolated specifically from the V depot of individuals with elevated BMI possess an enhanced intrinsic adipogenic potential, which is due, at least in part, to higher responsiveness of Akt to insulin stimulation.

OP 35 Health care delivery

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13-year post-trial follow-up of structured personal care for patients with type 2 diabetes in general practice

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Background and aims: In primary care, quality of diabetes care continues to be suboptimal. This 13-year post-trial study assessed the long-term effects of 6 years of structured personal diabetes care from diagnosis on mortality as well as cardiovascular and microvascular complications. **Materials and methods:** The study, Diabetes care in general practice, is a pragmatic, open, controlled trial with randomization of practices to structured personal care or routine care. In 1989–91, 474 volunteering Danish general practitioners (GPs) included 1263 patients with newly diagnosed type 2 diabetes. A further 106 intervention patients were included during the third year for the register-based follow-up only. The intervention included regular follow-up and individualized goal-setting supported by prompting of GPs, clinical guidelines, feedback, and education for GPs. Clinical follow-up was done 8 years after cessation of 6 years of intervention for 475 (86.2%) of 551 surviving of the 1263 patients. Predefined outcomes were all cause mortality and incidence of diabetic retinopathy, microalbuminuria, myocardial infarction, and stroke. Comparisons of the clinical observations between survivors in the intervention and routine care group were made using χ^2 -test for categorical variables and Wilcoxon non-parametric test for continuous variables. A register-based follow-up until death or censoring at 31 December 2008 with outcomes as in UK Prospective Diabetes Study included data from a further 5 years. Time from diagnosis to death or incident outcomes was analyzed with log-rank tests and Cox regression models (SAS PROC PHREG). All endpoints were analyzed according to the intention-to-treat principle.

Results: At clinical post-trial follow-up, relative risk reductions for diabetic retinopathy (25%, $P=0.044$) and microalbuminuria (28%, $P=0.023$) were observed. During 13 years of post-trial follow-up, risk reductions for myocardial infarction (18%, $P=0.038$) and any diabetes-related endpoint (15%, $P=0.029$) emerged.

Conclusion: These results indicate that individualized treatment goals as promoted by our multifaceted structured diabetes care program is a realistic and efficient alternative to using strict targets to treat patients in order to lower the risk of diabetic complications in the general population of type 2 diabetic patients.

Clinical Trial Registration Number: NCT01074762 (ClinicalTrials.gov)

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The National Diabetes Inpatient Audit 2010 (NaDIA) reveals concerns about inpatient care in English hospitals

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Background and aims: The prevalence of diabetes amongst people in hospital and the frequency of insulin use in this group are increasing in the United Kingdom. Surveys have revealed unacceptable inadequacies of inpatient diabetes care. The National Diabetes Inpatient Diabetes Audit (NaDIA) is a snap shot audit of bedside care inpatients conducted in a single day in 93% of English hospitals in November 2010. It aims to investigate and raise awareness of deficiencies in inpatient diabetes care as well as to drive and measure improvements in care overtime.

Materials and methods: 206 NHS hospitals, in 169 Trusts representing 93% of all Acute Trust in England took part. Bedside data was collected from 12,191 patients and 4745 completed a patient questionnaire.

Results: The Burden of Inpatient Diabetes People with diabetes accounted for 15.0% of audited beds. People with diabetes in hospital were older: Median age of 75 yrs compared to 68 yrs for all inpatients; People with diabetes have substantially longer length of stay: 8 nights compared with 5 nights for all ad-

missions; Nearly 40% were insulin treated. Delivery of diabetes care: Diabetes inpatient specialist input is low: 31.0%, 29.8% and 26.8% of units had no inpatient diabetes specialist nurses, diabetes dietetic or inpatient podiatry service respectively; Diabetes patients rarely see diabetes specialists: Few were under a diabetes consultant (9%) and 69.5% had not been seen by a member of the diabetes team. People with diabetes on insulin had poor glucose control: People with type 1 diabetes had 'good glucose days' (appropriate frequency of testing and values of 4–11 mmol/l) only one third of the time compared to just over half the time for patients with type 2 diabetes. There were significant issues concerning the use of insulin infusions: • 7.6% of insulin infusions were considered inappropriate • 9.9% of insulin infusions exceeded seven days; 12.0% were considered inappropriately long • In 25.8% the transfer to sub-cutaneous insulin was not managed appropriately. Medication errors were common and associated with poorer outcomes: • 26.0% of charts had prescription errors and 20.0% one or more medication management errors • Patients with medication errors had twice the rate of severe hypoglycaemia (18.1 vs 7.9%) • 0.4% (44) developed ketoacidosis and 2.4% severe hypoglycaemia (injectable treatment required) There are concerns about the prevention and management of diabetic foot disease • 21.4% of the units did not have access to a Multi-Disciplinary Footcare Team • Only 27.5% of patients had their feet examined • 2.2% developed a new foot complication during their stay

Conclusion: This unique snapshot audit reveals considerable concern about the care of inpatients with diabetes in most hospitals in England. The audit has now been adopted by the NHS as a driver for change to be repeated on an annual basis.

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Evaluating benchmarking to optimise management of type 2 diabetes patients: the final OPTIMISE study data for HbA_{1c}, LDL-cholesterol and systolic blood pressure in Belgium

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Background and aims: Type 2 diabetes is frequently associated with micro- and macrovascular complications which markedly impact on survival, quality of life and health care costs. Standards of care that include provision of effective drug therapies combined with lifestyle interventions reduce such burden and contribute to improved quality of care. Benchmarking incorporates two-sided feedback of physician's individual performance graded alongside the current mean achievement of a peer group, as well as patient's target attainment. This study aimed at assessing the effect of benchmarking on quality of care in type 2 diabetes outpatients over a 12-month period.

Materials and methods: Belgium is one among 6 European countries (BE, GR, LU, PT, SP, UK) participating in the non-interventional, observational OPTIMISE study. Physicians were randomly assigned using a cluster randomisation procedure to either a benchmarking group or control group. The primary endpoint was the percentage of patients achieving preset targets according to European guidelines (2007) for HbA_{1c} (<7%), low-density lipoprotein cholesterol (LDL-C <80 mg/dl Belgium, <100 mg/dl all other countries) and systolic blood pressure (SBP <130 mmHg). Follow-up (FU) markers of preventive screening, such as dietary counselling, smoking habits, physical activity, were also evaluated as secondary targets. The results presented here represent the Belgian final data.

Results: 95 GPs with 1142 patients were randomized to the intervention group and 95 GPs with 1036 patients to the control group. Both groups were highly-comparable regarding all baseline demographic and diabetes-related parameters. 65.0% (1414/2177) of patients were taking lipid-lowering drugs (predominantly statins: 93.4%, 1321/1414), 94.6% (2053/2174) were taking glucose-lowering medication(s), mainly biguanides (77.8%, 1599/2056), and 77.4% (1685/2178) were taking antihypertensives with a majority of patients (67.8%, 1143/1685) taking ≥2 medication classes. The frequency of patients achieving LDL-C target after 12 months FU was increased by benchmarking vs. control (41.1%, 392/953 vs. 35.3%, 319/900; p=0.011). A greater proportion of patients overall had high-density lipoprotein and total cholesterol levels considered excellent (reached target) in both groups after 12 months FU. The frequency of patients reaching SBP target was increased by benchmarking (41.7%, 332/797 vs. 30.5%, 214/701; p=0.003) but there was no significant difference in target HbA_{1c} attainment (62.1%, 605/975 vs. 63.8%, 594/931). The number of patients achieving all 3 targets (LDL-C, HbA_{1c}, SBP) increased after 12 months FU, and was higher (+75%) in the benchmarking group (8.6%, 84/975 vs. 4.9%, 46/931; p=0.001).

Conclusion: The final Belgian results of the OPTIMISE study indicate that benchmarking positively impacts combined target attainment for three major cardiovascular variables (SBP, HbA_{1c} and LDL-C) in type 2 diabetes mellitus patients. However, despite many patients being treated for these cardiovascular variables, the rate of control for LDL-C, SBP and HbA_{1c} remains sub-optimal.

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DiabetesE: Assessing progress in implementing national guidance on improving patient experience

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Background and aims: DiabetesE is an online self assessment tool which measures and benchmarks the quality of diabetes service provision and provides prioritised recommendations for service improvement. It aims to provide a means for encouraging and monitoring specialist diabetes teams' progress in implementing national guidance on improving patient experience.

Materials and methods: During 2010, assisted by an expert reference group of service users, clinicians and managers, a new DiabetesE questionnaire for specialist diabetes teams was developed, piloted and launched. Questions are grouped into 12 modules, including one called Patient Experience. This comprises 23 questions covering the provision, content and quality of structured education, care planning and user feedback, all based on national policy and guidance. A weighting and scoring system is applied to all modules and questions, enabling the automatic generation of scores and of prioritised recommendations for making service improvement. Users can update their answers and obtain revised scores and recommendations as soon as they have made changes to their services.

Results: Between the launch of the questionnaire in November 2010 and 31st January 2011, 97 specialist diabetes teams (community and hospital based) completed the Patient Experience module. The mean score was 71%, 91% of teams scored over 50% and 4% scored over 90%. Responses to questions relating to the NHS Operating Framework 2011/12 requirement that Primary Care Trusts should commission structured patient education to support people with diabetes show that 67% of participating teams offer structured education to people newly diagnosed with Type 1 diabetes and 82% offer ongoing education to people with diabetes under their care. In addition, 65% of teams provide structured education for people using insulin pump therapy, 81% of teams' patient education programmes have written, evidence based, structured curricula, and 86% of these programmes have specific aims and learning objectives. 60% of teams said that patient education programmes are assessed by independent assessors to ensure sustained quality and consistency but only 42% said that the education programmes have enough places to reflect the number of people newly diagnosed with diabetes. Responses to questions about the policy requirement to provide personalised care and care planning show that only 61% of participating specialist teams agree personal care plans with people newly diagnosed with diabetes. In addition, care plans are updated by just 51% of teams and a copy of the care plan is given to the patient by only 57%. Answers to questions about the need for services to regularly survey patients and seek their views to help improve patient experience reveal that whilst 73% of participating specialist teams actively survey people with diabetes about their experience of using the diabetes service, only 35% feed survey results back to patients and just 48% can demonstrate that they act on the results of patient surveys.

Conclusion: Although there is widespread provision of structured education and the majority of teams undertake patient experience surveys, there is considerable room for improvement to ensure that education programmes have the capacity to meet demand, patients are involved in care planning and are given feedback on the outcomes of surveys, and services act on the results of patient surveys. Ongoing use of DiabetesE will enable specialist teams to continually monitor their progress in implementing guidance on enhancing patient experience.

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Primary care providers: the key to improvement in diabetes care nationwide in Israel

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Background and aims: Clalit Health Services (CHS) is the largest health maintenance organization (HMO) in Israel, provides healthcare for 4 million citizens, 53% of the Israeli population, and 72% of patients with diabetes in Israel. Since 1995, the responsibility for management of diabetes was placed in primary care services. This study describes CHS's program "Diabetes in the Community" for improving diabetes care for all diabetes patients (300,000), insured by CHS nationwide.

Materials and methods: CHS operates 1,150 primary care clinics- all computerized since 2001, employing 4,000 general practitioners and family physicians, and 2,050 nurses. Multifaceted interventions were directed towards primary care providers and included an educational approach, registries, clinical pathways, care quality indicators, computerized reminders, patient empowerment and feedback. The quality of diabetes care was evaluated using diabetes-specific indicators. Initially, in 1995, performance was documented and evaluated manually. Computerized quality indicators for the entire diabetes patient load were available as of 2001, from which time data were collected by a computerized central system for all patients and were analyzed by SPSS for Windows, with the chi-square test for detecting statistical significance in categorical variables.

Results: The prevalence of diabetes, as reported to the registry, increased from 20.2/1000 in 1995 to 63.7/1000 in 2007. Annual performance of HbA_{1c} test rose from 22% in 1995 to 88% in 2007. LDL cholesterol test increased respectively from 23% to 89%, and microalbumin testing rose from 10% to 69% ($p<0.0001$ for all comparisons). The proportion of patients with HbA_{1c} $\leq 7\%$ rose from 10% in 1995 to 53% in 2007, while the proportion of patients with HbA_{1c} $> 9\%$ decreased from 40% to 13% ($p<0.0001$). The rate of LDL cholesterol ≤ 100 mg/dl rose from 26% in 2001 to 59% in 2007 ($p<0.0001$).

Conclusion: Primary care has a major role in diabetes care; charging it with the responsibility for diabetes care can lead to improvement of follow up and management of diabetes nationwide.

Performance of follow-up and control, Clalit Health Services diabetes patients, 1995–2007.

Indicator	1995	1997	1999	2001	2003	2005	2006	2007
HbA _{1c} performance	22.0%	45.0%	63.0%	70.0%	73.9%	83.3%	58.9%	87.9%
LDL performance	23.0%	38.0%	55.0%	67.6%	78.0%	83.0%	86.2%	88.7%
Microalbumin test	9.5%	22.7%	39.8%	39.0%	42.5%	56.9%	64.8%	68.9%
BP measurement	NA	NA	NA	NA	NA	NA	77.4%	87.5%
HbA _{1c} $\leq 7\%$	10.0%	33.0%	28.0%	35.0%	40.2%	43.4%	51.0%	52.7%
HbA _{1c} $> 9\%$	40.0%	37.0%	41.0%	25.0%	22.3%	17.0%	13.7%	13.0%
LDL ≤ 100 mg/dl	NA	NA	NA	26.4%	36.3%	46.7%	52.3%	59.1%
LDL > 130 mg/dl	NA	NA	NA	34.3%	28.4%	20.7%	17.6%	14.7%
BP $< 130/80$ mmHg	NA	NA	NA	NA	NA	NA	47.0%	52.0%
BP $> 160/90$ mmHg	NA	NA	NA	NA	NA	NA	10.4%	8.2%

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Changes in diabetes therapy and the quality of diabetes care in patients with type 1 diabetes 1989 to 2010: the JEVIN-Trial

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Background and aims: The JEVIN-Trial started in 1989/90 as a cross-sectional survey on the quality of diabetes care of insulin treated diabetes patients ($n=190$; age 16–60y.) who were registered in Jena. The cohort was examined every 5 years. In 2004/05 and 2009/10 the follow up was continued only for patients with type 1 diabetes. Since the beginning of the trial enormous changes in social structure and medical care and therapy have occurred. The

health care system was decentralized in 1989/90 and structured treatment, as well as teaching programmes for intensified insulin therapy were established. After 1995 privately run diabetes specialized out-patient care was established, human insulin replaced animal insulin, insulin analogues were introduced, use of insulin pump increased and Point-of-Care-Testing diagnostic with multiple daily blood glucose controls became standard. The aim was to analyze changes in diabetes therapy and quality of diabetes care in a closed population of patients with type 1 diabetes in a period of 20 years.

Materials and methods: Of 131 patients with type 1 diabetes examined in 1989/90, 104 (79.4%) patients could be detected in 2009/10, 23 (17.6%) had deceased, 13 (9.9%) refused to participate. 68 (84%) patients were examined: age 59.4y; women 35.3%, diabetes duration 36y, BMI 26.6kg/m², blood pressure 144/82mmHg. Mean A1c-values are DCCT adjusted (normal mean 5.05%).

Results: 98% of the patients used conventional insulin therapy in 1989/90, after 2004/05 all patients used basal/bolus insulin regimes or insulin pump therapy. The frequency of blood glucose self-monitoring increased from 2.5/week in 1989 to 35/week in 2010 ($p<0.001$). In 1989/90 only animal insulin therapy was used, in 2000 78.6% had human insulin, 11.9% human- + analogue insulin, 9.5% animal- + human- + analogue insulin. In 2010 analogue insulin was used in 37.3%, human insulin in 35.8%, human- + analogue insulin in 26.9%. A1c-values in 1989 were 7.5% ($n=63$), 1995 8.4% ($n=52$), 2000 7.5% ($n=42$), 2005 7.7% ($n=48$) and 2010 6.9% ($n=67$). The increase in A1c from 1989 to 1995 ($p=0.007$) and the decrease from 1995 to 2000 ($p<0.001$) as well as from 1989 to 2010 ($p=0.008$) are significant, but not the A1c-values from 1989 to 2005. The prevalence of retinopathy was 69%, peripheral neuropathy 51% and albuminuria 39%.

Conclusion: During the follow-up of the long term study from 1989 to 2010 the political and social setting was completely turned over and the structure of diabetes treatment and therapy in patients with diabetes type 1 changed enormously. Centralized diabetes care and conventional therapy with animal insulin was replaced by decentralized diabetes care and intensified therapy with analogue- and human insulin. In spite of all changes, the quality of diabetes care remained remarkably good and stable. After a temporary deterioration until 1995, the initial quality of care was obtained again after 10 years and even exceeded after 20 years.

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Effects of diet and diet plus physical activity on psychological outcomes in newly diagnosed type 2 diabetes mellitus: results of a randomised controlled trial

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Background and aims: The Early Activity in Diabetes (Early ACTID) Trial showed that both dietary and dietary plus activity interventions based on Motivational Interviewing improved glycaemic control, weight loss and reduced medication use over 12 months when compared to usual care in patients with newly type 2 diabetes mellitus (T2DM). Dietary advice alone was as effective as diet plus activity over this period. Few studies have reported the effect of diet and exercise on psychological outcomes, particularly soon after diagnosis or compared the benefits of increased physical activity over and above intensive dietary support on these outcomes. Here we aimed to determine the effect of increased physical activity on the psychological outcomes over that produced by intensified dietary intervention or usual care in individuals with newly diagnosed T2DM.

Materials and methods: 593 individuals, aged 60±10 yrs and 197±58 days from diagnosis were randomly assigned in a 2:5:5 ratio to receive usual care (UC, n=99; initial dietary consultation and 6 monthly follow-up), dietary (D, n=248; 3 monthly dietary consultation with monthly nurse support) or dietary plus activity (DA, n=246; equal contact time as D but with addition of a pedometer based exercise programme). The Diener's Satisfaction with Life Scale, EQ-5D scale, Diabetes Treatment Satisfaction and Brief Illness Perception Questionnaires were completed at baseline, 6 and 12 months.

Results: At baseline there were no statistically significant differences in illness perception, treatment satisfaction or EQ-5D between the three groups. Patients in UC had a lower Diener's score than D (22±7 vs 24±6 p=0.001), and DA (22±7 vs 24±7, p = 0.006) at baseline, but there were no differences between D and DA. UC only saw an improvement in their understanding (6.7±2.3 vs 7.4±2.1, p=0.012), expectation of disease continuation (9.0±2.2 vs 9.5 ± 1.9, p=0.028) and concern of their illness (5.3±3.0 vs 4.7±3.2, p=0.018) across the 12 months. D increased their understanding (6.9±2.4 vs 8.0±1.8, p <0.001), became less concerned (5.7±3.0 vs 5.1±3.0, p=0.005) and felt more in control of their illness (7.1±2.3 vs 7.6±2.0, p=0.002) over the 12 months. Satisfaction with treatment (29±6 vs 32±4, p<0.001) and self reported health scores (73±17 vs 76±16, p=0.007) also improved. DA showed similar improvements to D with the exception of their health scores which did not improve (74±16 vs 76±16, p=0.115). Neither intervention groups showed improvements in satisfaction with life. At 12 months, D and DA, compared to UC, felt in greater control of their illness (7.6±2.0 (D) vs 6.7±2.5 (UC), p=0.002; 7.6±2.0 (DA) vs 6.7±2.5 (UC), p <0.001), were more satisfied with their diabetes treatment (32±4 (D) vs 31±5 (UC), p=0.002; 32±4 (DA) vs 31±5 (UC), p <0.001) and had higher self reported health scores (76±16 (D) vs 71±17 (UC), p=0.047; 76±16 (DA) vs 71±17 (UC), p=0.013). No differences in any of the measures were seen at 12 months between D and DA.

Conclusion: D and DA programmes introduced soon after diagnosis of T2DM help patients to understand and feel more in control of their illness, as well as improving their satisfaction with treatment and health status. They do not however improve satisfaction with life and adding activity to a diet programme seems to confer no additional benefit.

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Higher number of stressful life events in families of children with type 1 diabetes mellitus onset under 5 years of age than in families of healthy controls

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Background and aims: Recent years show the worldwide increase in incidence of T1DM in every age group. There hasn't been found one good explanation

why the biggest increase is seen in children at the age 0-4 old. Stress is one of factors seen as contributing to T1DM development. The aim of this study was to assess the incidence rate of serious life events in families of children with T1DM prior to diabetes onset and examine if there is a difference in occurrence of such events depending on the age of a child at T1DM recognition in comparison with families of children in general population.

Materials and methods: The study was carried out from November 2008 to June 2010. There were included 822 families: 347 parents of children with T1DM and 475 parents of children without diabetes. Participants with T1DM were divided into two groups depending on the age of diabetes diagnosis: parents of 123 parents of children (67 girls, 56 boys) with T1DM onset at the age 5 years while the control group consisted of parents of 124 children (64 girls, 60 boys), at the age 0-5 years, and parents of 350 children (172 girls, 178 boys) at the age 6-17 years. Parents of children with T1DM completed a questionnaire on stressful events during the routine control visit, parents in control group - during the parents meeting. The questionnaire contained a hierarchical list of stressful events which may cause Posttraumatic Stress Disorder.

Results: Families of children with T1DM onset <5ys had experienced serious life events before diabetes diagnosis significantly more often than control subjects 57/66 vs. 38/86 respectively, $\chi^2=6.43$, $p=0.011$. There was no difference in the number of families of children with T1DM recognized 5ys affected with serious life event 57/66 vs. 108/116 respectively, $\chi^2=0.94$, $p=0.369$, and between children with T1DM recognized >5ys and controls 108/116 vs. 173/177 respectively, $\chi^2=0.303$, $p=0.608$. Results of multiple logistic regression analysis of serious life events in families of children with diabetes onset at < 5 years of age showed that 2 events were significantly associated with diabetes: divorce (OR 3.31 95%CI 3.51to21.4, $p=0.002$) and serious accidents (OR 2.21 95%CI 1.02to81.92, $p=0.048$).

Conclusion: Children with diabetes recognized < 5ys were affected more often with serious life events before diabetes diagnosis than their healthy peers which highlights the possible association between stress in families and the development of type 1 diabetes in the youngest children. Especially parental loss due to divorce may be a factor in the development of diabetes. Further prospective studies should be carried out to expand understanding of involved mechanisms.

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The course of depression in primary care patients with type 2 diabetes: results from the DiaDDZoB study

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Background and aims: Compared to controls without diabetes, the risk of depression is doubled in individuals with type 2 diabetes, affecting approximately one in every five patients. Despite a growing body of literature demonstrating the health risks of depression in this patient group, the course of depression has received relatively little attention. Therefore, the main aim of the present study was to examine the incidence and recurrence/persistence of depression in primary care patients with type 2 diabetes and to identify significant predictors of these course patterns.

Materials and methods: A cohort of 2460 primary care patients with type 2 diabetes was assessed for demographic, clinical and psychological factors, including symptoms of depression, in 2005 (baseline) and followed-up in 2007 (M_1) and 2008 (M_2). Incidence and recurrence/persistence of depression were defined using data from these three separate assessments, with a score of ≥ 12 on the Edinburgh Depression Scale (EDS) indicating depression. Stepwise logistic regression analyses were used to determine whether incident and recurrent/persistent depression could be predicted by means of baseline (1) demographics (female sex, age, being single, low education); (2) medical co-morbidities (prior cardiovascular disease, the presence of microvascular complications, a history of other co-morbid conditions); (3) stressful life events, self-reported history of depression (only for recurrence/persistence).

Results: Prevalence rates of depression were 12%, 14% and 16% at baseline, M_1 and M_2 , respectively. A total of 2124 patients completed the EDS at one or more assessments, with 21% reporting an EDS-score of ≥ 12 at least once (any depression). Compared with men, women were more likely to be depressed at any moment during the study (28%, $n = 303$ versus 15%, $n = 152$; $p < 0.001$). In the subgroup with no self-reported history of depression and no baseline depression ($n=1675$), incident depression at M_1 or M_2 was present in 171 individuals (10%), with a higher rate for women (13%, $n = 109$ versus 7%, $n = 62$; $p < 0.001$). As for recurrent/persistent depression, 46% of the 222 patients who were depressed at baseline also met the criterion for depression

at M_1 and/or M_2 . Recurrence rates were similar for female and male patients (48%, $n = 72$ versus 42%, $n = 30$; $p = 0.458$). Female sex (OR 2.35, 95% CI 1.45–3.81), low education (OR 1.87, 1.10–3.16), microvascular disease (OR 1.78, 1.13–2.79), other co-morbid conditions (OR 1.67, 1.06–2.62) and stressful life events (OR 1.70, 1.09–2.67) were all positively associated with incident depression, while the cardiovascular co-morbidities increased the odds of depression by 28%, but did not reach statistical significance ($p = 0.300$; $n = 971$). Low education level was the only significant predictor of recurrent/persistent depression (OR = 2.69, 95% CI 1.01–7.15; $n = 133$).

Conclusion: Depression is common in primary care patients with type 2 diabetes and often presents as a chronic condition. As recognition rates of depression are generally low while effective antidepressant treatments are available, monitoring of emotional well-being seems warranted, but should be embedded in collaborative care approaches.

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Atlantic DIP: Diabetes in Pregnancy: a comparative study of stress and wellbeing in women with established diabetes, gestational diabetes, and those without diabetes

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Background and aims: Diabetes in pregnancy increases the risk of maternal and perinatal morbidity and mortality. The experience of diabetes during pregnancy may be a significant source of stress, both because of the impact of the illness and associated treatments on the expectant mother and because of concern about the impact on the unborn child. In order to examine stress associated with diabetes during pregnancy, we carried out a prospective study in women with pre-existing (Type 1 or Type 2) Diabetes (PDM), Gestational Diabetes Mellitus (GDM), and non-diabetic pregnant controls (NDM).

Materials and methods: The participants were 210 pregnant women - 25 with pre-existing diabetes (PDM), 77 with GDM and 108 healthy controls (NDM). All were attending antenatal services in six health care centres in Ireland. We measured stress and wellbeing with several standardised psychological questionnaires including The Pregnancy Experience Scale; The Depression Anxiety Stress Scale; the Multidimensional Perceived Social Support Scale; the Illness Perception Questionnaire-Diabetes; the Diabetes Self-Efficacy Scale; the SF-8 and the Problem Areas in Diabetes Scale. We hypothesized that diabetic women would report higher levels of stress than healthy controls and we also hypothesized that social support may confer a protective role.

Results: We found a non-significant trend of increased stress and lower quality of life among diabetic women compared to non-diabetic controls. Women with PDM perceived their illness as having a higher impact on their lives than those with GDM ($p < 0.0001$). However, women with pre-existing diabetes also reported significantly greater self-efficacy in relation to their diabetes management compared to their gestational diabetes counterparts ($p < 0.05$). The results of the remaining questionnaires demonstrate a general trend towards higher distress in diabetic women compared to controls. The healthy controls reported higher perceived social support which may confer a protective role against psychological stress.

Conclusion: These preliminary results suggest that pregnant diabetic women perceive themselves as having a lower quality of life and higher levels of stress in pregnancy, especially women with pre-existing diabetes. This may indicate a need for psychological support in these patients. However, further research is required.

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Comparing the effect of 2 different methods of follow-up after structured group education on psychosocial measures in patients with type 1 diabetes: the Irish DAFNE Study

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Background and aims: This study compared group follow-up (intervention) with individual clinic follow-up (control) after delivery of the Dose Adjustment for Normal Eating (DAFNE) education programme to 437 participants

with type 1 diabetes. Psychosocial measures were included as secondary outcomes in this cluster randomised controlled trial. Group follow-up did not impact on the primary outcome, change in HbA_{1c} over time. Rates of severe hypoglycaemia did not differ significantly between intervention and control groups but there was a significant improvement in rates of severe hypoglycaemia over time.

Materials and methods: Hospital Anxiety and Depression Scale (HADS), Problem Areas in Diabetes (PAID), and Diabetes-Specific Quality of Life Scale (DSQOLS) were used to assess psychosocial outcomes at baseline and at 6, 12 and 18 months post-DAFNE. HADS measures mood disorder in particular anxiety and depression. PAID assesses diabetes-related distress. A validated English language version of DSQOLS was used to measure quality of life. The instrument has 4 subscales, Social Aspects, Fear of Hypoglycaemia, Dietary Restrictions and Physical Complaints as well as an overall quality of life (QoL) score.

Results: Participants in both the group follow-up ($n = 216$) and the individual follow-up ($n = 221$) arm of the study reported a mean reduction in anxiety (HADS-Anxiety, -0.53, $p < 0.001$), in depression (HADS-Depression, -0.44, $p < 0.001$), in diabetes-related distress (PAID, -9.41, $p < 0.001$) and a mean increase in overall QoL (DSQOLS, 9.23, $p < 0.001$) respectively from baseline to 18 months. There was no significant difference between groups for any of the measures (HADS-Anxiety, 0.13, $p = 0.43$; HADS-Depression, 0.13, $p = 0.37$; PAID, 1.55, $p = 0.12$; DSQOLS, -1.95, $p = 0.08$). A mean improvement in both groups was also observed for each of the DSQOLS subscales with Social Aspects, Fear of Hypoglycaemia and Physical Complaints improving on average by over 7%, Dietary Restrictions improved on average by over 17% in both groups. Similar to the other psychosocial measures there was no treatment effect between groups.

Conclusion: All the measures reported improvements post-DAFNE, these initial improvements were maintained in quality of life, depression, anxiety and diabetes-related distress after 18 months. DAFNE aims to give people the skills necessary to efficiently match the correct insulin doses to the amount of carbohydrate eaten while emphasising dietary freedom and flexibility. This may account for the Dietary Restrictions subscale on average showing the greatest improvement over time. While this trial confirms that DAFNE improves initial psychosocial outcomes and helps maintain them over 18 months, it also shows that structured group follow-up provides no additional benefit over traditional one-to-one clinic visits.

Clinical Trial Registration Number: ISRCTN79759174

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Widespread decreased white matter tract integrity in type 1 diabetes mellitus patients with microangiopathy and its relation to cognitive functions and disease variables

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Background and aims: Type 1 diabetes mellitus (T1DM) is associated with brain volume loss and cognitive changes predominantly found in domains concerning information processing speed. A recent study, in a small sample, showed focal loss of white matter tract integrity in parts of the corpus callosum in T1DM patients relative to controls. This is of interest as loss of white matter tracts may result in loss of processing speed. Therefore, we aimed to identify changes in tract integrity in a large group of T1DM patients with and without microangiopathy and controls and determine its relationship with disease variables and cognitive functions.

Materials and methods: Forty-eight T1DM patients with and 52 patient without microangiopathy and 49 controls underwent MRI, including diffusion tensor imaging (DTI) with 60 directions of encoding. Using the diffusion toolbox and tract-based-spatial-statistics (TBSS), part of FSL4.1 software, we calculated fractional anisotropy (FA), as a marker of tract integrity. Cognitive functions were assessed using an elaborate cognitive test-battery. Analyses were corrected for age, gender, depressive symptoms and multiple comparisons.

Results: T1DM patients with microangiopathy were significantly older, had higher HbA_{1c} levels and depression scores compared to their counterparts and controls (all $P < 0.05$). Decreased FA was found in T1DM patients with microangiopathy relative to patients without microangiopathy and controls,

and was widespread, most notably in the bilateral inferior fronto-occipital fasciculus (IFO), corticospinal tracts, parts of the corpus callosum, cingulate gyrus, and in parts of the left temporal cingulate tract, whereas patients without microangiopathy showed a small, but statistically significant, decrease in FA in the frontal part of the brain compared to controls. As the left IFO showed the most consistent decrease in integrity in T1DM patients compared to controls we selected the mean FA-value of this tract for each participant, to determine correlations between FA of the left IFO, disease variables and cognitive functions. In the total population of T1DM patients, the mean FA-value of the left IFO correlated negatively with disease duration ($r=-0.338$) and albumin-to-creatinine ratio (ACR) ($r=-0.287$) and positively with general cognitive ability ($r=0.258$), information processing speed ($r=0.281$), executive functions ($r=0.280$) and attention ($r=0.301$) (all $P<0.01$).

Conclusion: T1DM patients with microangiopathy showed notable and widespread decreases in white matter integrity compared to controls and patients without microangiopathy, most pronounced in the left IFO and corticospinal tracts. In the T1DM patients, a reduced integrity of the IFO was associated with worse cognitive performance, longer disease duration and higher ACR levels. Longitudinal follow-up should identify possible mechanisms involved in decreasing FA and its relation to cognitive functional changes over time.

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OP 37 Diabetes and cancer

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The relationship between distribution of body mass index and all fatal and non-fatal cancers combined: the FINRISK study

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Background and aims: Although a high body mass index (BMI) has been well recognised to be associated with increased risk of all-cause mortality, fatal and non-fatal cardiovascular disease (CVD), its role as a risk factor for cancer events including fatal or non-fatal cancers remains controversial. The objective of this study was to investigate the relationship between BMI and cancer events based on the FINRISK study.

Materials and methods: We examined the association between cohort-specific BMI decile and cancer events in a prospective cohort study among 26 690 Finns aged 24–74 years, free of CVD, diabetes or cancer at enrollment. Multivariate-adjusted hazard ratios and 95% confidence intervals for cancer events were estimated using Cox proportional hazard analysis compared to the lowest BMI decile.

Results: During a mean follow-up of 15.9 years, 2 729 all fatal and non-fatal cancers combined occurred, including 880 fatal cancers and 2 611 non-fatal cancers. Crude risk (/1000 person-year) for all fatal and non-fatal cancers combined increased with increasing cohort-specific BMI decile, from 4.20 in the lowest BMI decile to 8.81 in the highest BMI decile in men and from 4.68 to 8.61 in women, respectively (P for linear trend both <0.001) (Figure 1). However, the linear relationship disappeared and HRs were not significant both in men and women, compared with the lowest BMI decile after adjusting for age, area, smoking status, leisure time physical activity, educational level, household income, alcohol consumption, daily intake of vegetable and fruit. The negative relationship remained after exclusion of fatal and non-fatal cancers combined occurring during the first five years of follow-up. Furthermore, the results for fatal or non-fatal cancers were similar.

Conclusion: Elevated BMI didn't increase the risk of cancer events after accounting for confoundings.

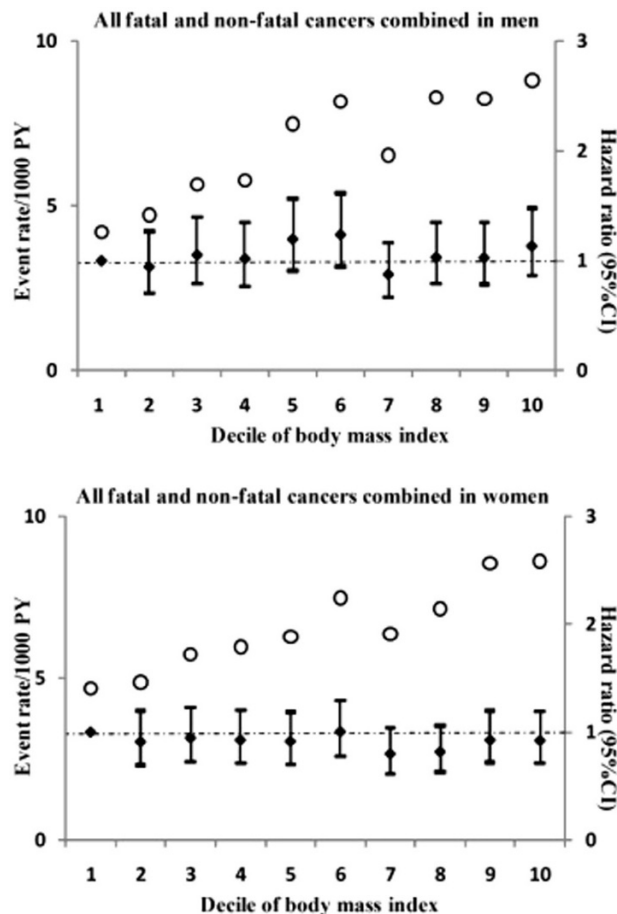


Figure 1 Crude events rate/1000 person-year (PY) (open circles), hazard ratio (solid diamonds) and 95% confidence interval (95% CI) (vertical bars) for all fatal and non-fatal cancers combined by deciles of cohort-specific body mass index (BMI) among men and women in FINRISK study adjusted for all confoundings at baseline. The lowest decile of cohort-specific BMI served as a referent group.

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Time-varying risk of breast cancer after onset of type 2 diabetes: evidence of detection bias in post-menopausal women

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Background and aims: There is evidence of a modestly elevated risk of breast cancer in women with type 2 diabetes. However, it is unclear whether this risk is related to a potential detection bias surrounding diabetes onset, and if it is limited to post-menopausal women. Our objective was to examine the risk of breast cancer in pre- and post-menopausal women with incident type 2 diabetes during earlier and later time windows following diabetes onset.

Materials and methods: This was a population-based retrospective cohort study, using linked health databases from British Columbia, Canada. From 1996–2006, we identified incident diabetes and non-diabetes cohorts of women, matched on age and index year. Following a minimum two-year cancer washout period, first breast cancers were identified prospectively in both cohorts, via linkage to the British Columbia Cancer Agency database, with follow-up until death, departure from the province, or March 31, 2006. Cox regression analyses were used to estimate the hazard ratios (HR) and 95% confidence intervals (CI) for the association between incident diabetes and breast cancer, adjusting for age, socioeconomic status, and frequency of physician visits in the two years prior to cohort index. We stratified this analysis, using age as a proxy, for pre- (<55 years) and post-menopausal (≥55 years) status. We examined this relationship in earlier (0–3 months) and later (3 months–10 years) time windows after diabetes onset.

Results: There were 84,506 women in each of the diabetes and matched non-diabetes cohorts and the mean (SD) age was 61.8 (14.2) years. Mean (SD) follow-up was 4.4 (2.9) and 4.5 (2.9) years for the diabetes and non-diabetes cohorts, respectively. Over the full follow-up period, breast cancer incidence rates (per 1,000 person-years) were 1.83 (95%CI: 1.61–2.08) and 1.92 (95%CI: 1.68–2.21) for pre-menopausal women with and without type 2 diabetes, and 4.02 (95%CI: 3.77–4.29) and 3.89 (95%CI: 3.62–4.19) for post-menopausal women with and without type 2 diabetes, respectively. In the first 3 months following diabetes onset, the HRs for breast cancer were 0.95 (95%CI: 0.48–1.86; $p=0.88$) and 1.31 (95%CI: 0.92–1.86; $p=0.14$) in pre- and post-menopausal women with type 2 diabetes, respectively, compared to women without type 2 diabetes. In the later time window of 3 months to 10 years following diabetes index, the risks of breast cancer were 0.92 (95%CI: 0.75–1.13; $p=0.45$) and 1.00 (95%CI: 0.90–1.11; $p=0.93$) in pre- and post-menopausal women with type 2 diabetes, respectively.

Conclusion: Our findings suggest a trend towards an increased risk of breast cancer in post-menopausal women with type 2 diabetes, but only in the time period immediately following diabetes onset. These observations suggest previous reports of an increased risk of breast cancer in women with diabetes may have been over-estimated due to detection bias at the time of diabetes onset.

Supported by: CIHR

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The influence of glucose-lowering therapies on cancer specific mortality in type 2 diabetes

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Background and aims: Following the general concern regarding the association between various diabetes treatments and cancer risk, the present study evaluated the cancer specific mortality in patients with type 2 diabetes.

Materials and methods: All type 2 diabetes patients residing in a major urban area, with onset over 40 years and receiving diabetes treatment were

included at the moment of their first diabetes outpatient visit from 1st January to 31st December 2001. All patients were followed-up for death of any cause until 31st December 2009 by crosslinking with the National Institute of Statistics mortality database, with a virtually 100% cover-up. Mortality data for the general population was obtained from the same source. Death related data was based on death certificate. Demographics and diabetes history were obtained from the Regional Diabetes Registry and patient files. Antidiabetic prescriptions for baseline and follow-up period, including the dose were available from local pharmacies or prescribing institutions. Glitazones, GLP-1 analogues, DPP4 inhibitors and basal insulin analogues were not marketed in 2001. A total of 21473 subjects (45.1% males) were divided into four groups according to whether they received monotherapy with biguanides (BIG, $n=4123$, 19.2%), insulin secretagogues (SEC, $n=8008$, 37.3%), combined oral therapy (COM, $n=7345$, 34.2%), or insulin (INS, $n=1997$, 9.3%) at baseline. Adjusted Cox proportional hazard models and other (non)parametric tests were used for statistics.

Results: At baseline, mean age was 64.06 ± 9.75 years and mean diabetes duration 5.89 ± 6.36 years. Mean follow-up was 7.4 ± 2.42 years (158808.2 person-years). Overall, unadjusted all-cause mortality was 43.4/1000 person-years (6893 deaths); cancer mortality was 7.25/1000 person-years (1152 deaths). The major types of cancers were: pulmonary ($n=179$, 15.5%), colorectal ($n=175$, 15.2%), pancreatic ($n=114$, 9.9%), liver ($n=109$, 9.5%), breast ($n=102$, 8.9%) and prostate ($n=59$, 5.1%). All-cause and cancer specific mortality rates in the general population were 11.18/1000 and 2.05/1000 subjects respectively. Age adjusted cancer mortality rates for 1000 person-years were calculated using the general population as reference: overall 2.76 (CI95% 2.6–2.92), BIG 2.15 (CI95% 1.83–2.47), COM 2.23 (CI95% 1.99–2.47), SEC 3.22 (CI95% 2.94–3.5) and INS 4.12 (CI95% 3.39–4.85). Comparing with MET, the adjusted HR for cancer mortality was 1.08 (CI95% 0.9–1.3, $p=0.38$) for COM, 1.47 (CI95% 1.24–1.75, $p<0.001$) for SEC and 1.59 (CI95% 1.27–2.01, $p<0.001$) for INS. In INS group, future exposure to basal insulin analogues during follow-up did not changed the cancer mortality for both glargine (HR 0.59 CI95% 0.29–1.21, $p=0.15$) and detemir (HR 0.21 CI95% 0.03–1.55, $p=0.13$). Compared with BIG, the INS group had a higher risk for liver (HR 3.59 CI95% 1.68–7.66, $p<0.001$) and breast (HR 2.28 CI95% 1.09–4.79, $p=0.03$), but not for pulmonary, colorectal, pancreatic or prostate cancer mortality.

Conclusion: Taking into account the differences in age distribution, type 2 diabetes patients treated with insulin or insulin secretagogues are at increased risk for all-cause and cancer mortality compared with those on biguanides monotherapy. Exposure to long acting analogues was not associated with increased cancer mortality in insulin treated subjects.

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Identification of molecular pathways involved in diabetes-associated cancers

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Background and aim: A growing body of evidence indicates that diabetes mellitus increases the risks of site-specific cancers of the bladder, endometrium, liver, colorectum, pancreas and breast. Adipocytes and their precursor cells, may provide key factors needed for tumor development, progression, or even enable tumor cell invasion. Nevertheless, whether metabolic alterations at the level of the adipose tissue may affect specific phenotypes of the cancer cells is still unclear. Thus, the aim of this study is to evaluate whether metabolic factors, such as glucose and free fatty acids, may affect the functions of adipocytes in promoting breast cancer cell growth.

Methods: 3T3-L1 cells, human adipocytes and their undifferentiated precursors were incubated in the presence or in the absence of different concentrations of glucose (5.5mM–25mM) or fatty acids (Oleate 0.5–10uM; Palmitate 10–100uM); upon treatment, the cells were washed and incubated with serum-free media for 8 h. Conditioned medium (CM) has been collected and added to serum-starved MCF7 and MDA-MB-231 breast cancer cells for different times. Next, we have analyzed cell proliferation by cellular count, cell cycle phases by cytometric determinations and intracellular pathways by Western Blot analysis. To identify the growth factors and cytokines released from the pre-adipocytes/adipocytes, we have used ELISA multiplex.

Results: We have obtained evidence that CM from 3T3-L1 and human adipocytes cultured in 25 mM glucose (high glucose) induced growth of MCF7 and MDA-MB-231 cells, in a time-dependent manner. In particular, CM from fully differentiated adipocytes was 2-fold more effective than CM from pre-adipocytes in inducing breast cancer cell growth. Cell cycle analysis by flow cytometry revealed that these changes were accompanied by reduced apoptosis. Moreover, adipocyte CM activated PI3K, MAPK and JAK/STAT pathways in breast cancer cells. Interestingly, the pre-incubation of adipocytes with 5.5 mM glucose, a concentration corresponding to normal fasting glucose levels in humans, significantly prevented their ability to induce breast cancer cell growth. At variance, the stimulation of adipocytes with palmitate or oleate in 5.5 mM glucose almost completely restored their growth promoting action (1,43 and 1,37 fold increase over basal, respectively) to levels similar to those observed for cells cultured in high glucose. Secretion of IGF-1, RANTES and IL-8 was higher in adipocytes than in pre-adipocytes and was enhanced by incubation with high glucose or fatty acids. We have also confirmed these results by RT-PCR analysis of IGF1, RANTES and IL-8 mRNA levels. Moreover, treatment of breast cancer cells with IGF-1R or cytokine inhibitors reduced the adipocyte CM effect on cell growth.

Conclusion: In conclusion, adipocyte-derived factors promote breast cancer cell growth by inhibiting apoptosis. This effect is more evident for factors released by adipocytes than by pre-adipocytes and is modified by glucose and fatty acids. IGF-1, RANTES and IL-8 could be good candidates in mediating growth-promoting actions of adipocytes on breast cancer cells.

OP 38 Adiposity and cardiovascular disease

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Function of cardiac adipose differentiation-related protein (ADRP) in the pathogenesis of diabetic cardiomyopathy

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Background and aims: Diabetic cardiomyopathy is characterized by intracellular lipid accumulation (steatosis) that generates lipotoxicity and eventually leads to cardiac dysfunction. The adipose differentiation-related protein (ADRP) is a lipid droplet-associated molecule expressed in many tissues including heart, although its pathophysiological function remains uncertain. We have found that cardiac ADRP expression is increased upon fasting in concert with lipid accumulation, whereas ADRP is decreased in diabetic hearts despite steatosis. In order to explore the pathophysiological function of cardiac ADRP, diabetes was induced in transgenic mice with heart-specific overexpression of ADRP and hearts were analyzed.

Materials and methods: Cardiac-specific ADRP-overexpressing (Tg) mice were generated using cDNA of mouse ADRP and enhanced green fluorescent protein under control of the myosin heavy-chain- α promoter. The founders were backcrossed with C56BL/6 for 7 generations and heterozygous transgenic (Tg) mice and their wildtype (Wt) littermates were studied. The mice were injected with streptozotocin to induce diabetes and hearts were analyzed 3 weeks afterward. Cardiac collagen was extracted for measurement, and fibrosis was evaluated by sirius-red staining. Cardiac gene expression profiles were analyzed using Affymetrix microarrays and the diabetic profile was compared between Wt and Tg hearts. Cardiac function was studied with ultrasonography.

Results: In the absence of diabetes, Tg mice accumulated numerous lipid droplets around clusters of mitochondria in cardiomyocytes, which contained 8 times more triglyceride compared to Wt hearts. With diabetes, interstitial fibrosis in Tg hearts was comparable to that in Wt hearts, despite greater steatosis in Tg than Wt hearts. However, diabetes-induced fresh collagen was diminished in Tg hearts. Cardiac contractile function was impaired 15–18% with diabetes in both Wt and Tg mice. Microarray expression analysis showed that diabetes altered (>2-fold) expression of 1094 genes in Wt hearts and 1404 genes in Tg hearts. In Wt hearts expression of a fatty acid-responsive gene HMG-CoA synthase 2 and collagen IX were increased, but matrix metalloproteinase (MMP)-15 was decreased with diabetes. In contrast, in Tg hearts expression of HMG-CoA synthase 2 was unaltered and collagen 1 was decreased, but MMP-3, -12 and -15 were increased with diabetes. In addition, expression of a potent antioxidant protein metallothionein I and glucose 6-phosphate dehydrogenase, which directs excess glucose into the pentose phosphate pathway, were increased with diabetes in Tg hearts.

Conclusion: ADRP appears to play a role in packaging lipotoxic fatty acids into lipid droplets, leading to induction of cardio-protective genes and inhibition of excess collagen production when overexpressed in diabetes. Thus, cardiac ADRP could be a target to ameliorate diabetic cardiomyopathy.

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Burden of cardiovascular disease in „metabolically benign“ obese compared with „insulin-resistant“ normal-weight individuals

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Background and aims: “Metabolically benign obese” people, despite their body-fat, display a favorable metabolic profile characterized by high levels of insulin-sensitivity, and a beneficial blood-pressure, metabolic and inflammation profile. However, it remains controversial whether this healthier metabolic profile is translated into a lower cardiovascular disease (CVD) risk compared with “insulin-resistant” normal-weight individuals.

Materials and methods: A total of 550 individuals without diabetes or baseline macrovascular complications were studied. Participants were clas-

sified by presence ($n=271$) or absence ($n=279$) of Metabolic Syndrome and by Body Mass Index (BMI; <25 Kg/m²=normal-weight, $n=177$; 25 – 29.9 Kg/m²=overweight, $n=234$; ≥ 30 Kg/m²=obese, $n=139$). Metabolic Syndrome was diagnosed with the NCEP-ATP-III criteria. Left ventricular functional capacity, myocardial structure and performance were assessed by ultrasound.

Results: Six-year CVD incidence (nonfatal myocardial infarction, stroke, congestive heart failure) was compared by obesity and metabolic status. Obesity or BMI were not associated with increased CVD risk (HR 0.88, 95%CI 0.41–1.32). Presence of Metabolic Syndrome conferred 2.5-fold higher CVD risk (HR 2.5, 95%CI 1.68–3.40). Overweight/obese “metabolically-benign” individuals had the lowest 6-year CVD risk (HR 1.12, 95%CI 0.35–1.33) compared with normal-weight “insulin-resistant” people (HR 2.33, 95%CI 1.25, 4.36, $P=0.007$). From the metabolic components studied, impaired fasting glucose (HR 1.5, 95% 1.23–1.92), hypertension (HR 1.1, 95% 1.03–1.47), dyslipidemia (HR 0.48, 95% 0.39–0.58) and central-obesity (HR 1.33, 95% 1.17–1.64) were all associated with increased CVD risk. Physical inactivity (<7.5 Metabolic Equivalent-hours/week, smoking, insulin-resistance (HOMA-IR <2.5), low-grade inflammation (high-sensitivity C-reactive protein ≥ 1.5 mg/dl, micro-albuminuria) were independently associated with CVD incidence.

Conclusion: In contrast to normal-weight insulin-resistant people, “metabolically benign” individuals show decreased CVD-risk in a 6-year follow-up study.

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Plasma omentin levels in relation to cardiac function in patients with type 2 diabetes and healthy controls: effect of pioglitazone versus metformin

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Background and aims: The adipokine omentin is highly and selectively expressed in visceral adipose tissue and its circulating levels are decreased in obesity and type 2 diabetes mellitus (T2DM). In this study, we assessed the relationships between plasma omentin levels and cardiometabolic variables in T2DM men and age-matched overweight healthy controls. Next, only in the T2DM men, the effects of 24-wk treatment with pioglitazone or metformin on plasma omentin levels were investigated.

Materials and methods: We studied 78 asymptomatic T2DM men, treated with glimipiride monotherapy, in whom inducible ischaemia was excluded by dobutamine stress echocardiography, and 14 age-matched controls at baseline. Then, the T2DM patients were randomly assigned to pioglitazone or metformin therapy. Plasma omentin levels were measured at baseline and after the 24-wk treatment. Measurements included fasting cardiometabolic variables, clamp-measured insulin sensitivity, as well as magnetic resonance imaging to quantify body fat compartments and cardiac function.

Results: T2DM men had a higher body mass index, systolic blood pressure, HbA1c, visceral fat and subcutaneous fat volumes and a lower M-value compared to controls (all $P<0.02$). Plasma omentin levels were lower ($P=0.008$) in T2DM men vs. controls. Previously, we reported in this cohort that diastolic function differs between DM2 en controls. At baseline plasma omentin showed a significant correlation with visceral fat tissue volume ($r=-0.269$, $P=0.009$), while the association with subcutaneous and pericardial adipose tissue volumes were only marginally statistically significant (both $P=0.09$). Furthermore, plasma omentin levels correlated positively with early peak filing rate, early deceleration peak and early deceleration mean (all $P<0.05$). Plasma omentin levels were correlated with M-value as well ($r=0.379$, $P=0.001$). Twenty-four week of pioglitazone, vs. metformin, resulted in higher plasma omentin levels in T2DM men ($P=0.01$), in the presence of similar glycaemic improvements.

Conclusion: Plasma omentin levels were decreased in asymptomatic T2DM compared to age-matched healthy males, and were positively associated with cardiac diastolic function, while a negative relationship was observed with body fat compartments. Furthermore, pioglitazone versus metformin therapy increased circulating omentin levels. Based on these findings, we hypothesize that circulating omentin levels may be a marker of cardiac risk in T2DM.

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Relation of changes in components of the metabolic syndrome to changes in fat depots in a cardiovascular prevention programme

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Background and aims: Prevention programmes designed to reduce cardiovascular risk, using lifestyle, medication or a combination, are likely to generate some of their beneficial effects through reductions in adiposity; more specifically *via* effects on ectopic fat depots. Therefore, we wished to explore the association between alterations in total and ectopic fat and changes in components of the metabolic syndrome in response to a multi-factorial prevention programme.

Materials and methods: We assessed the components of the metabolic syndrome (IDF consensus 2006) together with fat depots before and at completion of a twelve week family based multi-factorial cardiovascular prevention programme (the MYACTION programme) including lifestyle and medication changes. Eighteen patients with cardiovascular risk $>20\%$ over 10 years were included. Fat depots were determined from whole body MR imaging; and ectopic fat in liver, pancreas and tibialis anterior muscle were determined using MR spectroscopy.

Results: Participants were aged 68.5 (SD 4.4) years. Their weight was 78.9 (13.1) kg and total fat volume 29.3 (6.4) L at baseline. Weight was reduced by -2.5% (95% CI -4.0% to -1.0% , $p=0.002$) and total fat by -10.8% (95% CI -15.7% to -5.8% , $p=0.0006$). Marked reductions in subcutaneous fat, and ectopic fat, were observed in response to MYACTION (table). Robust regression analysis, showed change in triglycerides was most closely associated with change in hepatic fat ($r^2=0.57$, $p=0.002$). In contrast, change in fasting glucose was predicted by change in both subcutaneous and visceral fat (r^2 0.59, $p=0.006$). Blood pressure and HDL change were not associated with change in any fat depot.

Conclusion: A short cardiovascular prevention programme is associated with profound reductions in ectopic fat. Individual components of the metabolic syndrome were differently related to changes in specific ectopic fat depots. Our findings have implications understanding how ectopic fat deposition impacts on cardiovascular risk factors, and how interventions reduce risk. They may also have implications for how we best target lifestyle interventions.

Effects of the 12 week MYACTION cardiovascular prevention programme

Measure	Baseline	Percent change	p-value
Subcutaneous fat, L	20.5 (4.6)	-10.2 (-14.3, -6.0)	0.0002
Visceral fat, L	4.8 (1.8)	-13.3 (-22.0, -4.5)	0.0066
Hepatic fat, AU	2.3 (1.5, 7.4)	-41.3 (-59.0, -23.6)	0.0002
Pancreatic fat, AU	8.4 (2.1, 15.4)	-35.5 (-55.9, -15.1)	0.0031
IMCL tibialis anterior, AU	6.7 (3.8, 9.0)	13.4 (-7.2, 33.9)	0.18
Waist circumference, cm	99.3 (7.6)	-3.7 (-5.3, -2.3)	0.0001
Fasting glucose, mmol/l	6.0 (1.5)	-0.3 (-5.7, 5.0)	0.89
Systolic BP, mmHg	134 (15)	-2.6 (-8.3, 3.0)	0.33
HDL cholesterol, mmol/l	1.4 (0.3)	1.3 (-6.1, 8.6)	0.71
Triglycerides, mmol/l	1.08 (0.74, 1.58)	6.5 (-8.4, 21.4)	0.36

Effects on fat depots and components of the metabolic syndrome. Data are means (SD) or median (IQR) for baseline and means (95% CI) for percent changes (p-values from *t*-tests). IMCL, intramyocellular lipids; AU, arbitrary units.

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OP 39 Prediction of type 2 diabetes

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Physical activity, abdominal and general obesity and the risk of developing type 2 diabetes: a case-cohort study (InterAct)

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Background and aims: The independent and joint effects of physical activity, overall and central adiposity on incident diabetes risk in population based samples are largely unknown. We examined whether physical activity predicts incident diabetes independent of overall and abdominal adiposity and also the joint effect of physical activity, overall and abdominal adiposity on diabetes risk.

Materials and methods: Case-cohort study nested within the European Prospective Investigation into Cancer (EPIC) study including 12,403 verified incident diabetes cases and a randomly selected sub-cohort of 16,154 individuals prospectively followed for 12.3 years. Overall physical activity was assessed by self-report, categorised into 4 groups and validated against combined heart rate and movement sensing in an independent sample ($n=1,941$) across study locations. Adiposity was measured by BMI and waist circumference (waist circumference >94 cm and >80 cm in men and women, respectively). Hazard ratios per unit increase in PA were estimated separately within each centre/country using Prentice weighted Cox regression, with age as the underlying timescale and additionally adjusting for educational level, smoking status, alcohol consumption and energy intake. Hazard Ratios were combined across centres/countries using random effects meta-analysis, and the percentage of variation between centres/countries due to real heterogeneity was calculated (I^2).

Results: Over a median follow-up time of 12.28 years (192,876 total person years), 6.3% of men and 3.9% of women developed diabetes. Diabetes incident rates decreased by increasing levels of physical activity in men (5.78, 5.93, 5.36, 4.14 per 1000 person years) and in women (4.57, 3.03, 2.57, 2.54 per 1000 person years). A one level increase in overall physical activity was associated with a 12% (HR, 0.88, 95% CI 0.81;0.95) and 7% (HR 0.93, 95% CI 0.89;0.98) relative reduction in the risk of type 2 diabetes independent of BMI, study centre, education, smoking status, alcohol consumption and energy intake in men and women, respectively. Similar effect sizes were observed after further adjustments for waist circumference and when examining the associations between leisure time physical activity with incident diabetes in both men and women. In joint analyses, the risk of developing diabetes was significantly increased with lower levels of overall physical activity across BMI categories in men (p for trend <0.003) and women (p for trend <0.003), except for obese women (p for trend $=0.25$). The risk of developing diabetes increased by decreasing levels of overall physical activity in both abdominally lean (P for trend $=0.009$) and abdominally obese (P for trend $=2.5 \times 10^{-7}$) men and in abdominally lean (P for trend $=0.002$) and abdominally obese (P for trend $=10^{-4}$) women.

Conclusion: Overall physical activity is associated with reduced risk of developing type 2 diabetes independent of general and abdominal adiposity. Overall physical activity also reduces the risk across BMI categories and to a similar magnitude in both abdominally lean and obese men and women. Prevention of type 2 diabetes needs to consider both weight reduction and increasing levels of overall physical activity.

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Combination of FINDRISC and fasting plasma glucose (FPG) in screening for type 2 diabetes in an Irish population: the type 2 diabetes mellitus and vascular health initiative (DMVHI)

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Background and aims: Lifestyle modification in high risk and pre-diabetic individuals will prevent or delay development of T2DM. However, screening for T2DM is complex and expensive. To identify those at risk, the largest

Irish health insurer has undertaken a study to assess prevalence of undiagnosed T2DM and pre-diabetes by screening a subset of its 1.3 million policy holders (representing 62% of the insured population in Ireland). Our aims were to identify the prevalence of undiagnosed T2DM, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) in an Irish population and to develop criteria combining FINDRISC and FPG for use in population screening.

Materials and methods: All policy holders aged 45–75 years, without known diabetes, living within the catchment area of two Dublin University Teaching Hospitals were invited to participate. Participants completed a detailed medical questionnaire. FINDRISC, FPG, lipid profile, blood pressure, weight, height, BMI, waist and hip circumference were measured. Subjects with initial results in the IFG range ($FPG \geq 5.6 \leq 6.9$ mmol/L) had an OGTT. Multiple logistic regression was used to estimate the coefficients of FINDRISC and FPG in a model where T2DM was the outcome variable.

Results: Over 11,500 participants (56% women; 44% men) have been screened to date. The mean age was 60 ± 8.0 years. 21% required OGTT. The prevalence of T2DM, IFG, and IGT was 2.2%, 6.9%, and 3.4% respectively. A model was constructed combining the FINDRISC and FPG by adding 2 points per 0.1 mmol/L of FPG above 5.5 mmol/L to the FINDRISC score. The ROC AUC for the combined score was 0.818 (95% CI 0.780–0.854) versus AUC for FINDRISC alone of 0.675 (95% CI 0.632–0.718), $p < 0.001$. The new scoring system with a cutoff of 15 had 91% sensitivity for T2DM.

Conclusion: The combined FINDRISC and FPG score, as outlined above, would identify 91% of undiagnosed T2DM and 74% of pre-diabetic individuals for further investigation and reduce the number of OGTTs from 21% to 12% of the screened population. Our data suggests that this new scale could provide a practical and effective population screening tool.

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Excess diabetes in ethnic minorities - incidence and predictors

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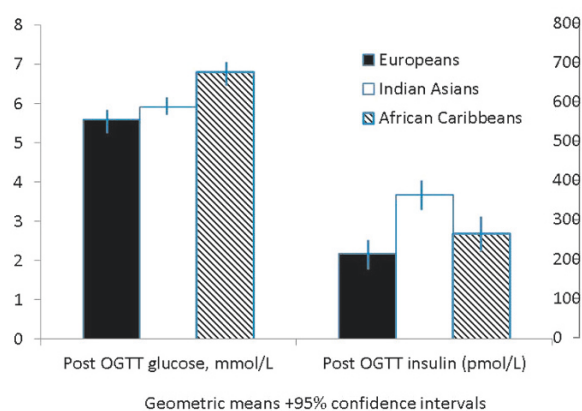
Background and aims: Excess prevalence of diabetes in people of Indian Asian (IA) and African Caribbean (AfC) descent is well known. However, risks of incident diabetes, diabetes in older age and predictors thereof are poorly understood. We addressed these questions in a tri-ethnic cohort.

Materials and methods: Population based sample of 2945 men and women (1457 European (E), 1070 IA and 418 AfC) aged 40–70 years and living in north west London. Baseline (1988–91) and follow-up (2008–2011) measures included fasting and post OGTT bloods, anthropometrics, blood pressure and health/lifestyle questionnaire. Follow-up also included primary care medical record review.

Results: Diabetes prevalence at baseline (mean age 52.3 ± 6.9) was 6% (E), 20% (IA) and 19% (AfC). After 20 years of follow-up (mean age 70.3 ± 6.3), prevalence was 19% (E), 45% (IA) and 41% (AfC). An additional 26% E, 20% IA and 18% AfC had pre-diabetes (IFG, IGT or both). Age and sex adjusted hazard ratios for ethnicity as a predictor of incident diabetes (Europeans=reference): IA: 2.50 (2.11, 3.03), AfC: 2.38 (1.86, 3.05). Adjustment for baseline risk factors (fasting blood glucose, insulin and lipids, blood pressure, occupational status, physical activity and obesity) partially attenuated the IA excess, but did not alter the AfC excess (fully adjusted HRs respectively: 1.94 (1.54, 2.45), 2.33 (1.72, 3.16)). However, baseline glucose and insulin relationships from the OGTT in those who developed diabetes differed markedly by ethnicity. Post OGTT glucose was significantly higher in AfC than in either E or IA. Post OGTT insulin was comparable in AfC and E, whilst in IA plasma post OGTT insulin was nearly doubled. (Figure).

Conclusion: Diabetes and pre-diabetes are astonishingly frequent in all older people, affecting around two thirds of British Indian Asians and African Caribbeans and nearly half of Europeans. Ethnic differences in incidence are unexplained by conventional risk factors measured 20 years earlier. Development of diabetes in African Caribbeans may be a consequence of a poor insulin secretory response to glucose. In contrast, Indian Asians who developed diabetes exhibited a markedly greater insulin secretory response to glucose, suggesting greater insulin resistance. Pathways to diabetes differ by ethnicity.

Baseline 2 hour post OGTT glucose and insulin in people who developed diabetes during follow-up



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Metabolic markers of insulin sensitivity predict progression to IGT and type 2 diabetes

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Background and aims: Insulin resistance (IR) is a risk factor for type 2 diabetes. An unmet medical need exists for a practical measurement of insulin sensitivity to identify high-risk, IR patients for more effective lifestyle intervention and disease prevention. We previously identified biomarkers of insulin sensitivity in a cross-sectional metabolic profiling analysis of a nondiabetic, healthy population (n=399), RISC, an observational, prospective study, based on their highly significant correlations to the euglycemic clamp glucose disposal rate.

Materials and methods: Using UHPLC-mass spectrometry with absolute quantitative measurements of the top-ranking predictors of insulin sensitivity, we describe their incorporation into an algorithm developed from an expanded analysis of the entire RISC cohort (n=1277) and validate their predictiveness of dysglycemia on the independent Botnia Prospective cohort.

Results: One model (IR algorithm), incorporating insulin, alpha-hydroxybutyrate (AHB), linoleoylglycerophosphocholine (L-GPC), oleate, and BMI, resulted in AUCs of 0.86 and 0.85 for identifying the bottom quintile and bottom tertile of insulin sensitivity, respectively (p<0.001). Using 3-year follow-up data in RISC, we observed that our IR algorithm was statistically equivalent to the clamp's glucose disposal rate in predicting conversion from NGT to IGT (AUC=0.70, n=880). In a separate cohort, to assess the clinical utility of the top-ranking insulin sensitivity markers identified in the RISC study, we carried out a targeted analysis of subjects followed in the Botnia Prospective Study (n=2585). In this family history type 2 diabetes cohort, we measured said analytes and IR algorithm and compared T2D progressors to non-progressors. Based upon multiple logistic regression analysis, L-GPC and AHB was significantly associated with diabetes progression, independent of sex, age, family history diabetes, BMI, and basal FPG, with an odds ratio of 0.66 (C.I.: 0.523–0.832) and 1.28 (C.I.: 1.09–1.51, p=0.0029), respectively, for each SD of their targeted plasma measurements. In a 5-year timeline, using leave-one-out cross-validation, L-GPC and AHB had AUCs of 0.68 (p=6.93 e-6) and 0.65 (p=0.0002), respectively, for predicting T2D progression, whereas our multi-analyte IR algorithm had an AUC of 0.78 (p=1.049 e-11) when removing insulin, and an AUC of 0.82 (p=2.934 e-15) when adding age, sex, and FPG.

Conclusion: We demonstrate that measurement of a panel of insulin sensitivity markers in a fasting plasma sample can identify high-risk, IR subjects with high accuracy, and predict progression to IGT and T2D.

OP 40 Transplantation

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Achievement of good long-term glycaemic control after islet transplantation: single or multiple initial islet transplantations?

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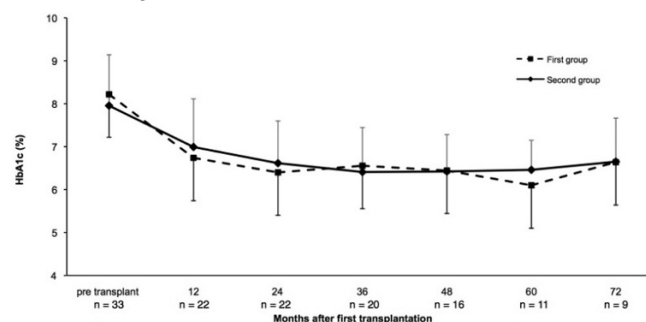
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Background and aims: The main goal of islet transplantation in patients with type 1 diabetes mellitus has changed over the last years from insulin independence to good glycemic control and avoidance of severe hypoglycaemia. However, no data exists about the optimal initial islet mass. The aim of this study was to compare posttransplant outcome in patients with different initial islet mass.

Materials and methods: Patients who underwent combined islet-kidney or islet after kidney transplantation at our institution with multiple initial islet transplantations (11 patients with maximal 5 transplants, 1st group) as compared to single initial islet transplantation (22 patients who were retransplanted only if good metabolic control was no longer achieved, 2nd group) were included in this study. Patients were seen before and every three months after transplantation to assess posttransplant glycemic control and to adjust insulin therapy if necessary.

Results: The time between the first and second transplantation was 2.4±2 and 25.8±9 months, respectively. There was no significant difference in baseline characteristics in the two groups: Age was 50.8±8.5 and 53.6±7.4 y with 45.5% and 72.7% male patients, diabetes duration of 38.1±7.8 and 37.4±11.3 y. BMI was comparable at baseline (22.6±1.8 and 24.6±5.1 kg/m²). Total follow-up was 78 and 51 months. Islet retransplantation was performed 0.35 times per patient-year of follow-up in the 1st group and 0.11 times in the 2nd group. HbA1c decreased significantly in both groups after transplantation (p=0.017 and p=0.026) but did not differ between the groups during follow-up (Figure). Frequency of severe hypoglycaemia (need for assistance, loss of consciousness) was comparable in the two groups after transplantation (0.16 vs 0.13 hypoglycaemia/patient-year, p=0.88). Insulin dosage decreased significantly in the 1st group (from 0.62 IE/kg to 0.25 IE/kg, p=0.017) during the first year after transplantation, but not in the 2nd group (from 0.54 IE/kg to 0.4 IE/kg, p=0.38). Similarly, C-peptide was higher one year after transplantation in the 1st group (1454±554 pM) as compared to 655±443 pM (p=0.028) in the 2nd group (single transplants).

Conclusion: This study demonstrates that a single initial transplantation with retransplantation only if glycemic control deteriorates, uses less pancreas donors as compared to multiple initial transplantations, but results in equal glycemic control and rate of severe hypoglycemic episodes despite a higher demand of exogenous insulin.



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Sustained improvement of cardiovascular risk factors (CVRF) and cardiac function in type 1 diabetic (TD1) patients with successful pancreas transplant alone (PTA)

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Background and aims: The role of PTA in the treatment of T1D patients (pts) is still debated. Aim of this study was to investigate the course of several CVRF and cardiac function in T1D after PTA, comparing pts with functioning or failed pancreas graft.

Materials and methods: We studied 47 T1D pts (age: 36 ± 8 yrs, BMI 23.3 ± 2.8 kg/m², diabetes duration 23 ± 10 yrs) who underwent PTA (72% portal-enteric drainage, tacrolimus- and MMF-based immunosuppression) and were followed for 6.4 ± 1.9 yrs. Clinical and laboratory assessments were performed by standard procedures and doppler echocardiographic examinations were accomplished with the Sonos 5500 Agilent echograph.

Results: During the follow-up (FU), 35 pts (74.4%, group A) maintained PTA function (normoglycemia without exogenous insulin therapy), whereas in 12 pts (25.6%, group B), the graft failed. The two groups did not differ for any major clinical pretransplant (preTx) feature. Compared to preTx, the functioning PTA (FU: 6.3 ± 1.8 yrs) established sustained insulin and C-peptide secretion (3.6 ± 2.2 vs 0.08 ± 0.12 ng/ml, $p < 0.01$), normalized HbA1c (5.6 ± 0.4 vs $8.5 \pm 1.7\%$, $p < 0.01$) and improved total cholesterol (170 ± 33 vs 200 ± 45 mg/dl), LDL-cholesterol (93 ± 29 vs 125 ± 43 mg/dl), fibrinogen (330 ± 65 vs 370 ± 83 mg/dl) and diastolic blood pressure (76 ± 10 vs 81 ± 10 mmHg) (all $p < 0.01$), without significant changes in statin or antihypertensive therapy. In addition, ultrasound measured ejection fraction (EF) of heart left ventricle increased from 55.2 ± 2.9 to $58.1 \pm 2.0\%$ ($p < 0.01$). Successful PTA did not affect triglycerides, HDL-cholesterol and systolic blood pressure. In group B, PTA functioned for 0.35 ± 0.16 yrs, and parameters were analysed 6.7 ± 2.0 yrs after graft loss, with withdrawn of immunosuppressive therapy. In these pts, all the above parameters were similar preTx and at the last FU control. In both groups creatinine levels increased similarly (A: 1.18 ± 0.32 vs 0.92 ± 0.23 mg/dl, +28%, $p < 0.01$; B: 1.06 ± 0.31 vs 0.83 ± 0.17 mg/dl, +27%, $p < 0.01$).

Conclusion: Successful PTA restores long-term endogenous insulin secretion and, hence, normoglycemia, also leading to improvements of several CVRF and left ventricle ejection fraction; in our series, the decline of kidney function was similar in patients with and without functioning PTA, suggesting that the nephrotoxicity of long-term immunosuppression was balanced by the overall beneficial effects of normoglycemia.

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Successful clinical translation of intramuscular islet autotransplantation

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Background and aims: Intra muscular transplantation (IMT) offers attractive prospects for islet transplantation. The clinical relevance of this non invasive alternative to the intraportal route is not clearly established. We developed in the minipig a simple and reproducible technique for islet IMT and tested its clinical relevance for islet autotransplantation.

Materials and methods: Islets were isolated from adult minipigs (n=25) with standard automated technique following distal pancreatectomy. Standardized autologous islet grafts were implanted in the thigh gracilis muscle. Transplant sites were explanted and analyzed by immunochemistry for cell composition (insulin, glucagon) and vascularization (vWF). A similar technique was applied in two patients who underwent islet autotransplantation in the left forearm after an extended pancreatectomy for benign tumors.

Results: In the minipig, immunochemistry confirmed the presence of alpha and beta cells up to 6 months after transplantation. Islets revascularization increased between day 15 and day 30 and was correlated with islet survival. Graft function was documented by acute insulin response (AIR) after completion of the pancreatectomy at one month in 2 animals. Clinical islet IMT was uneventful in both cases. Ectopic insulin secretion could be documented by AIR with or without forearm vascular exclusion, and represented 53% and 22% of the overall systemic insulin secretion, respectively. Islet survival was confirmed by scintigraphy with radiolabeled GLP1 analogs after one year in our first patient.

Conclusion: These results provided unequivocal proof of prolonged survival, revascularization and function of pancreatic islets after IMT and confirmed the clinical relevance of this strategy for islet autotransplantation.

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Culture and injection of desferrioxamine (DFO) improves islet transplant outcomes

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Background and aims: Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor which co-ordinates a program of cellular response to stressors including hypoxia. Islet transplantation subjects islets to hypoxic environments and it is thought that up to 70% of islets die within 1 week of transplantation. We hypothesised that increasing HIF-1α would improve outcomes in adequate mass islet transplants and that treating recipient mice would further improve outcomes.

Aim: To investigate the effects of increasing HIF-1α protein in human islets upon islet-transplant outcomes.

Materials and methods: Isolated human islets were cultured overnight in control media, or media supplemented with desferrioxamine (DFO), a small-molecular stimulator of the HIF-1α protein. For each donor, at least 1 transplant was performed for each group, thus avoiding inter-donor variability. Islets were transplanted into diabetic SCID mice. Adequate mass model: Isolated human pancreatic islets from 12 separate donors were treated in the following groups:

- 1) Supra-physiological-mass transplant of 2000-control IEQ (islet equivalent)
- 2) 2000-DFO- islets were cultured overnight with DFO prior to transplantation and
- 3) 2000-DFO+injection- where islets were cultured with DFO and recipient mice additionally received DFO on days 1, 2, 4 and 7 post-transplantation. Mice were followed for 28 days post-transplantation and then underwent nephrectomy to confirm diabetes recurrence. In a subset of mice (4 donors), the graft was removed at 24 hours to assess apoptosis.

Results: Recipients of 2000 control IEQ achieved normoglycaemia in 74% of cases. 2000-DFO transplants were successful in 73% of mice and 2000-DFO+Injections in 92% of cases. Considering only successful transplants, recipients of DFO treated islets had significantly better random-fed glucose levels ($p = 0.03$ by ANOVA for repeated measures). Similarly, DFO+Injection mice had significantly better random-fed glucose levels than controls ($p = 0.02$). On glucose tolerance testing 25 days post-transplantation, a significantly higher proportion of mice receiving islets cultured with DFO had normal glucose tolerance ($p < 0.05$). Apoptosis 24 hours post-transplantation was decreased in both DFO groups by 30–50%. Beta cell mass was 40–50% greater at 28 days post transplantation in both DFO groups.

Conclusion: Glucose control post transplantation was improved by DFO. The mechanism is decreased short-term apoptosis leading to increased β-cell mass at 28 days. This data demonstrates that increasing HIF-1α in human islets in the pre- and peri-transplant period improves outcomes. HIF-1α and DFO may have a therapeutic role in human islet transplantation.

Supported by: NHMRC, DART, JDRF

OP 41 Retinopathy: experimental

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Diabetes or high glucose-induced apoptosis involves mitochondrial fragmentation in retinal pericytes

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Background and aims: Previously we have reported that high glucose (HG) induces mitochondrial fragmentation and promotes apoptosis in retinal endothelial cells, a critical step in the pathogenesis of diabetic retinopathy. In this study, we examined if HG induces mitochondrial fragmentation by altering expression of Fis1, a mitochondrial fission protein, in bovine retinal pericytes (BRPs). Additionally, the proapoptotic event was verified in diabetic human retinal pericytes.

Materials and methods: To assess if HG induced changes in mitochondrial morphology and Fis1 expression, BRPs were grown for 7 days in normal (5 mM) or HG (30 mM) conditions, stained with MitoTracker Green, a mitochondria-specific dye, and digitally-captured images analyzed for Aspect Ratio (AR) and Form Factor (FF); Fis1 expression was determined by Western blot analysis and cells undergoing apoptosis was assessed by TUNEL assay. To determine temporal effects of HG on mitochondrial morphology, a time course study was performed at 0.5, 1, 2, 6, 24, 48 hours and 7 days and mitochondrial morphology assessed through AR and FF analyses. In addition, human retinal pericytes isolated from age-matched normal, non-diabetic (NHuRP) and diabetic (DHuRP) septuagenarian donor eyes were analyzed for mitochondrial morphology.

Results: Significant increase in cells with fragmented mitochondria was observed in BRPs grown in HG (FF=3.00 compared to 4.71 in normal, $p<0.001$; AR = 2.54 compared to 2.92 in normal, $p=0.006$; $52.8\pm13.6\%$ of cells with fragmented mitochondria vs $22.3\pm10.4\%$ in normal, $p=0.004$) and DHuRPs compared to respective controls (35% of cells with fragmented mitochondria vs 13% in normal). Increased mitochondrial fragmentation was concomitant with increased apoptosis in BRPs grown in HG (5.6 ± 2.6 TUNEL-positive cells/1000 cells compared to 13.6 ± 3.5 TUNEL-positive cells/1000 cells in HG, $p=0.007$). A bimodal pattern in mitochondrial fragmentation was evident after 30 minutes of HG exposure, followed by recovery of the elongated normal mitochondrial morphology after 6 hours, continuing up to 48 hours (30 minutes: FF=3.16 compared to 4.88 in normal, $p<0.001$; AR=2.47 compared to 2.92 in normal, $p=0.015$; 6 hours: FF=4.35, $p<0.001$; AR=2.95, $p=0.03$). After initial recovery, mitochondrial fragmentation recurred and remained permanent. Importantly, Fis1 was significantly upregulated in BRPs following 7 days of HG exposure ($162\pm29\%$ of normal, $p<0.05$).

Conclusion: The findings indicate that diabetes promotes mitochondrial fragmentation in retinal pericytes, at least in part, through overexpression of Fis1 and plays an important role in mitochondrial dysfunction associated with accelerated cell death. Thus, mitochondrial fragmentation could represent a novel therapeutic target for preventing retinal pericyte apoptosis in diabetic retinopathy.

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Retinal adenosinergic system is affected by diabetes/hyperglycaemia

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Background and aims: Diabetic retinopathy exhibits characteristics of chronic inflammation. It is known that in diabetes there is an activation of microglial cells and a concomitant release of inflammatory mediators that cause death of retinal neurons. Microglial cells express all four types of adenosine receptors (A1, A2A, A2B, A3) and it is known that activation of A2A and A3 receptors in these cells leads to inhibition of pro-inflammatory cytokines expression and release and therefore could exert some protective effects on diabetic retinopathy. However, it is still unknown if this adenosinergic system is affected by diabetes/hyperglycemia (D/H). In this work we have studied the effect of diabetes or hyperglycemia, considered the main cause of diabetes complications, on this system in the retina.

Materials and methods: In this study we have used two cellular models: 1-retina cells, isolated from newborn Wistar rats, and cultured in control

(5mM glucose) or in high glucose (30 mM glucose) conditions. Cell cultured in media containing 5 mM glucose and 25 mM mannitol were used as an osmotic control; 2-whole retinas, obtained from control or STZ-induced diabetic Wistar rats. The effect diabetes or hyperglycemia has on adenosine receptors and adenosine deaminase (ADA) protein levels were evaluated by western blotting. The activity of ADA was evaluated by a enzymatic assay based on the Bertholet reaction and the viability tests were performed by the MMT assay.

Results: We have found that in retina cells, cultured in high glucose conditions (30 mM), the protein levels of A1 and A2A adenosine receptors are increased, up to $119.1\pm6.4\%$ and $192.5\pm19.9\%$ of control (5 mM glucose) whereas A2B and A3 protein levels were not different from control or mannitol cultures. In retinas, isolated from STZ-induced diabetic rats, we have found that A2A and A3 receptors protein levels were increased after one week of diabetes induction ($218.4\pm11.1\%$ and $150.6\pm11.7\%$ of control, respectively). Beside the effect on adenosine receptors, we have also found in retina cells, cultured in high glucose conditions, that although the protein levels of ADA were not different from control or osmotic control ($p>0.05$) the enzyme activity was significantly reduced to $24.4\pm6.5\%$ of control ($p<0.001$). We have already observed that there is a decrease in cell viability in retinal cells subjected to hyperglycemic environment (30 mM glucose). We have now found that this decrease in cell viability (to $90.0\pm3.3\%$ of control, $p<0.05$) was prevented by application of CGS 21680 (30 nM), an A2A adenosine receptor agonist ($96.7\pm1.9\%$ of control, $p>0.05$).

Conclusion: In this work we have found that D/H affects the retinal adenosinergic system. We have found that in D/H there is an increase in the protein levels of A2A receptors in both cellular models used. Since activation of this receptor subtype protects cells from high glucose induced cell death, it is tempting to propose that the transient increase in the expression of A2A receptors could function as a protective mechanism against D/H effects of retinal cells. The extent and implications of such changes are yet unclear, but if proven relevant in the context of DR, it would mean that extracellular adenosine dynamics and receptors could be aimed as new targets for the therapy or prevention of DR.

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The contribution of NADPH oxidase (Nox)1 and 4 to diabetic retinopathy: role of microglia

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Background and aims: Reactive oxygen species (ROS) are elevated in diabetic retinopathy and are implicated in cellular damage. The nicotinamide adenine dinucleotide phosphate-oxidase (Nox) enzyme is a major source of ROS production; however, the contribution of Nox to diabetic retinopathy is not fully understood. We aimed to determine if deletion of Nox1 and Nox4 isoforms influence the expression of angiogenic and inflammatory mediators involved in the development of diabetic retinopathy. As Nox expression is influenced by angiotensin II, and blockade of angiotensin II has benefits for neurovascular injury in diabetic retinopathy, we evaluated whether type 1 angiotensin receptor blockade (ARB) reduces retinal Nox expression. Finally, we evaluated which retinal cell types express Nox1 and Nox4.

Methods: Nox1 and Nox4 knockout (KO) mice and their wildtype (WT) controls were non-diabetic or made streptozotocin (STZ) diabetic and studied for 20 weeks. Sprague Dawley rats were randomized to be non-diabetic, STZ diabetic or diabetic+ARB (valsartan, 40mg/kg/day, gavage) and evaluated for 4 weeks. Primary rat retinal microglia, rat retinal ganglion cells, rat retinal glia, bovine retinal pericytes and bovine retinal endothelial cells were studied.

Results: WT diabetic mice showed significant increases in retinal VEGF and ICAM-1 mRNA levels compared to non-diabetic controls (1.4 fold and 2.5 fold mRNA respectively, $P<0.05$). In comparison, diabetic Nox1 KO and Nox4 KO mice had reduced levels of retinal VEGF and ICAM-1 mRNA compared to diabetic WT ($P<0.05$). In diabetic rats, retinal Nox4 expression was increased 2.7 fold compared to non-diabetic control ($P<0.05$), and was reduced to control levels with ARB. In vitro, microglia, the resident immune cell of the retina, most abundantly expressed Nox1 (50 fold increase) and Nox4 (100 fold increase) compared to retinal neurons, endothelial cells, glia and pericytes ($P<0.05$). Cultured microglia expressed components of the renin-angiotensin system including the angiotensin type 1 receptor. In diabetic rats,

ARB reduced the expression of the microglial activation markers, CD11b and major histocompatibility complex class II to control levels ($P < 0.05$).

Conclusion: In diabetic retinopathy, abundance of Nox1 and Nox4 is increased in retina, a phenomenon associated with augmented expression of angiogenic and pro-inflammatory factors. The abundance of Nox1 and Nox4 in retinal microglia, and the finding that ARB protects against the activation of retinal microglia, demonstrates that microglia may play a key role in ARB-mediated protective effects in diabetic retinopathy.

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Inhibition of endoplasmic reticulum stress delays diabetic cataract formation and prevents retinal oxidative stress and apoptosis

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Background and aims: Evidence for the important role for endoplasmic reticulum (ER) stress in diabetes is emerging, but its contribution to diabetic complications is poorly explored. The purpose of this study was to evaluate the role for this phenomenon in diabetic cataract formation and retinal oxidative stress and apoptosis using a pharmacological approach with two chemical chaperones counteracting ER stress *in toto*, trimethylamine-N-oxide (TMAO) and 4-phenylbutyric acid (PBA).

Materials and methods: Control and streptozotocin-diabetic rats were maintained with or without TMAO (110 mg kg⁻¹d⁻¹, in the drinking water) or PBA (100 mg kg⁻¹d⁻¹, i.p.), for 12 weeks starting from induction of diabetes (a prevention paradigm). Diabetic rats received suboptimal doses of insulin to prevent ketoacidosis and weight loss. Lens clarity was evaluated by indirect ophthalmoscopy and slit lamp examination on weekly basis. Cataracts were scored (0 - clear lenses, 1-vacuoles, 2-opacities, 3-mature cataract). At the end of the study, the rate of apoptosis was assessed by the number of TUNEL-positive nuclei in the flat-mounted retinae. The expression of retinal ER stress variables including total and phosphorylated PKR-like eukaryotic initiation factor 2A kinase (PERK), total and phosphorylated inositol-requiring enzyme-1 (IRE1), and CAAT/enhancer-binding protein homologous protein (CHOP), was measured by Western blot analysis. The indices of oxidative-nitrosative stress, 4-hydroxynonenal (HNE) adducts and nitrotyrosine (NT), were evaluated by ELISA.

Results: All the lenses were clear 2 wks after induction of diabetes. During the next 6 wks, TMAO slightly delayed diabetic cataract formation, with significant differences in cataract scores for the 5th wk only (0.667 ± 0.114 vs 0.944 ± 0.151 in untreated diabetic group, $p < 0.05$). The anticataract effect of PBA was more potent, and was observed till the end of the study, with significant differences in the cataract scores for the 10th, 11th, and 12th wks. Rats with 12-wk STZ-diabetes displayed ER stress, manifest by reduced phospho-PERK/PERK ratios, with unchanged phospho-IRE1/IRE1 ratio and CHOP expression. A chemical chaperone treatment increased retinal phospho-PERK/PERK and phospho-IRE1/IRE1 ratios and reduced CHOP expression, this potentiating unfolded protein response and counteracting ER stress. A 4.3-fold increase in retinal apoptosis in diabetes was completely prevented by TMAO, and essentially prevented by PBA. Both chemical chaperones counteracted diabetes-induced retinal HNE adduct and NT accumulation.

Conclusion: ER stress is implicated in diabetes-induced cataract formation, and retinal oxidative-nitrosative stress and apoptosis. The findings identify a new therapeutic target for diabetic ocular complications.

OP 42 Postprandial metabolism

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A prospective longitudinal observational study on the effect of dietary sugar intake in adolescence and early adulthood on measures of glucose metabolism in early adulthood

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Background and aims: There is conflicting evidence concerning carbohydrate type, insulin resistance and the development of diabetes. The increased consumption of the westernised diet in society, which is high in refined sugar and fat, has led to increased rates of obesity, insulin resistance and type 2 diabetes mellitus. The effect of the high sugar intake in adolescence is less well demonstrated. We examined the effect of sugar intake in early teenage years on glucose tolerance and insulin resistance in early adulthood.

Materials and methods: A detailed dietary history was performed in subjects aged 12 - 15y between 1989-1990 and again in early adulthood aged between 20-25y between 1997-1999. A total of 489 subjects returned for assessment in early adulthood and had an oral glucose tolerance test, from which the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated. Univariate analysis was performed to assess the relationship between dietary sugar intake and outcome measures with adjustment for sex, height, weight, BMI, social class, physical activity level, total calorie intake, total fat intake and skinfold thickness.

Results: In early teens mean dietary proportions of carbohydrate, fat and protein were $52 \pm 0.2\%$, $39 \pm 0.2\%$ and $11 \pm 0.1\%$ and mean total daily sugar intake (expressed as % of total energy) was $22 \pm 0.3\%$. Mean fasting plasma glucose in early adulthood was 4.4 ± 0.02 mmol/L, median fasting serum insulin level was 10.0 mU/L (Interquartile range 8.0, 14.0) and median HOMA-IR score was 2.0 (Interquartile range 1.5, 2.9). There was no association between dietary sugar intake in early adulthood and measures of glucose metabolism in early adulthood. There was a linear relationship between sugar intake in adolescence and fasting plasma glucose in adulthood with a 0.01 mmol/L increase for every % increase in dietary sugar $p = 0.04$. There was also a linear relationship between dietary sugar intake in adolescence and HOMA-IR score in early adulthood with a 2% increase in the HOMA-IR score for every % increase in dietary sugar ($p < 0.01$).

Conclusion: Higher dietary sugar intake in early teenage years independently affects longer term glucose metabolism and is associated higher fasting plasma glucose and relative insulin resistance. These findings indicate a potential legacy effect of the adolescent diet and a need to address dietary advice in this age group.

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Postprandial glucose metabolism in carriers of TCF7L2 risk allele with abnormal glucose regulation (AGR)

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Background and aims: The mechanisms responsible for contribution of common variants in the gene TCF7L2 to the risk of developing Type 2 diabetes remains far from being completely understood. In order to gain better insights in the metabolic implications of this genotype, studies were undertaken to evaluate postprandial glucose metabolism in subjects with AGR (i.e., IGT and/or IFG) carrying the TCF7L2 risk allele.

Materials and methods: Fourteen subjects (age: 59 ± 10 years; BMI 28 ± 4 kg/m²) with risk-conferring TCF7L2 genotypes (TT or CT at rs7903146) and 8 subjects (age 54 ± 11 ; BMI 28 ± 4) with wild-type genotype (CC) underwent a 4-hr meal-tolerance test with [6,6-(3)H]glucose infusion and [U-13C] glucose ingestion to study endogenous glucose production (EGP) and gut glucose absorption. All subjects had AGR as determined by OGTT. Glucose metabolism was determined along with measurements of plasma concentra-

tions of insulin, C-peptide and glucagon. Insulin secretory profiles were determined by C-peptide deconvolution and mathematical modeling.

Results: There was no difference between individuals carrying the risk genotype and the control group with respect to basal levels of glucose (99 ± 3 vs. 93 ± 4 mg), insulin (12 ± 1 vs. 13 ± 2 mU/l), C-peptide (3.4 ± 0.1 vs. 3.5 ± 0.1 ng/ml), and endogenous glucose production (EGP: 13.6 ± 2.2 vs. 18.1 ± 3.1 $\mu\text{mol/Kg/min}$), whereas plasma glucagon was lower in the risk allele carriers (62 ± 4 vs. 93 ± 9 pg/ml; $p=0.002$). In response to meal ingestion there was no difference in glucose concentration although the risk allele carriers had reduced insulin secretion rates during the 1st and the 2nd hour ($p<0.05$). No difference, however, emerged with respect to dose-response, and potentiation. Average EPG during the meal test did not differ between the 2 groups (4.4 ± 1.1 vs. 7.1 ± 2.1 $\mu\text{mol/kg/min}$). In contrast, AUC for total rate of appearance (Ra) tended to be lower in TT/CT (4016 ± 1503 $\mu\text{mol/min}$ vs. 7304 ± 4180 $\mu\text{mol/min}$) mainly due to significantly reduced oral Ra (3225 ± 1396 $\mu\text{mol/min}$ vs. 5888 ± 2877 $\mu\text{mol/min}$, $p=0.04$). In the whole population oral glucose Ra correlated with mean PG following the meal ingestion ($r=0.44$; $p=0.07$). Plasma glucagon levels remained constantly lower in risk carrying subjects (97 ± 11 vs. 60 ± 4 pg/ml; $p=0.018$) accounting for slightly greater insulin:glucagon ratio (1.09 vs. 0.85).

Conclusion: Individuals with abnormal glucose regulation carrying the rs7903146 TCF7L2 risk genotype do not have significant alteration in glucose tolerance after the ingestion of a mixed meal in spite of impaired insulin secretion rate. This impairment may be compensated by lower glucagon levels and reduced rate of appearance of oral glucose.

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Assessment of diurnal variations of insulin sensitivity, beta cell responsivity and hepatic insulin extraction in normal subjects

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Background and aims: Assessing whether postprandial glucose metabolism follows a diurnal pattern could be of great help in the development of an Artificial Pancreas. However, such variations in post prandial insulin action, secretion and hepatic extraction have never been studied with state of the art methods, in healthy or in type 1 diabetic subjects. The aim of present study was thus to determine the presence/absence of a diurnal pattern in insulin sensitivity (S_i), beta-cell responsivity to glucose (Φ) and hepatic insulin extraction (HE) in response to identical mixed meals in normal individuals.

Materials and methods: We studied 14 nondiabetic subjects (7 men, BMI 25.1 ± 1.0 kg/m², age 29.6 ± 2.0 yrs) with normal fasting glucose (4.8 ± 0.1 mM) and HbA_{1c} ($5.3 \pm 0.1\%$), who received, on three consecutive days, three identical mixed meals (10 cal/kg, 50 g carbs; 35% carbs, 30% protein, 35% fat) either for breakfast (B), lunch (L) or dinner (D) at 0700, 1300 and 1900 in randomized latin square order. Physical activity measured with accelerometers was equal on all three days. Plasma glucose, insulin and c-peptide concentrations were frequently sampled during each test. Oral glucose, c-peptide and insulin minimal models were applied to provide estimates of S_i , dynamic Φ (Φ_d), static Φ (Φ_s), overall Φ (Φ_{tot}), basal (HE_b) and total HE (HE_{tot}).

Results: S_i was higher at B than L and D, but not significantly ($S_i^B=10.41 \pm 2.11$, mean \pm SE, $S_i^L=7.82 \pm 0.90$, $S_i^D=7.45 \pm 0.82$ 10^{-4} dl/kg/min per $\mu\text{U/ml}$); Φ_d was significantly lower at L compared to B and D ($p=0.004$ and $p=0.042$, respectively; $\Phi_d^B=695.17 \pm 62.95$, $\Phi_d^L=527.32 \pm 75.59$, $\Phi_d^D=625.15 \pm 103.26$ 10^{-9}); Φ_s was significantly lower at L compared to B ($p=0.035$; $\Phi_s^B=42.67 \pm 4.19$, $\Phi_s^L=38.21 \pm 3.39$, $\Phi_s^D=38.43 \pm 3.95$ 10^{-9} min⁻¹); Φ_{tot} was significantly lower at L and D compared to B ($p=0.017$ and $p=0.042$, respectively; $\Phi_{tot}^B=54.24 \pm 4.89$, $\Phi_{tot}^L=45.74 \pm 4.54$, $\Phi_{tot}^D=47.39 \pm 5.70$ 10^{-9} min⁻¹); HE_b was significantly higher at D compared to B ($p=0.010$; $HE_b^B=0.69 \pm 0.02$, $HE_b^L=0.72 \pm 0.03$, $HE_b^D=0.74 \pm 0.03$) and total HE was significantly higher at L and D compared to B ($p=0.040$ and $p=0.004$, respectively; $HE_{tot}^B=0.48 \pm 0.03$, $HE_{tot}^L=0.62 \pm 0.03$, $HE_{tot}^D=0.54 \pm 0.03$).

Conclusion: Our results demonstrate the existence of diurnal pattern in insulin action, secretion and extraction in healthy humans. This is represented by a progressive decrease in insulin sensitivity and beta-cell function with a concomitant increase in hepatic insulin extraction from B to D. Similar studies in type 1 diabetes are currently underway.

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Effect of age, sex, mitochondrial function and exercise training on insulin sensitivity, beta cell responsivity and fatty acid concentrations in response to a mixed-meal

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Background and aims: It remains to be determined whether the effect of specific exercise programs on muscle mitochondrial function is related to improvement in insulin sensitivity. We determined the effects of different exercise programs in both young and elderly adults on skeletal muscle mitochondrial function and insulin sensitivity, beta cell responsivity, and free-fatty acid (FFA) concentrations.

Materials and methods: We studied the effects of 8 wk of endurance (ET), resistance (RT), and combined (CT) training on meal tolerance and muscle oxidative capacity in 34 young (25 ± 1 y, 46% female) and 31 elderly (70 ± 2 y, 50% female) adults. Participants ingested a mixed-meal (25 kcal/kgFFM: 50:30:20, CHO: FAT:PRO), followed by serial (20-min) blood draws for 2h to measure glucose, insulin, c-peptide, and FFA. Measures of insulin sensitivity (S_i) and beta-cell responsivity (dynamic Φ_d , static Φ_s and total Φ_{tot}) were calculated using the oral glucose and c-peptide minimal model. FFA concentrations were measured by HPLC. Mitochondria were isolated from muscle biopsies before and after training. State-3 O_2 flux was measured with substrates for complex I, I+II, and II by high-resolution respirometry.

Results: At baseline there were no significant differences in S_i between men and women, nor the young and the elderly (all $p>0.05$). However, men had higher baseline Φ_d and Φ_{tot} than women ($\sim 30\%$, $p=0.046$; $\sim 17\%$, $p=0.051$, respectively) and the young had higher baseline Φ_d and Φ_{tot} than the elderly ($\sim 40\%$, $p=0.010$; $\sim 30\%$, $p=0.051$, respectively). Although no significant increase in S_i or Φ occurred following exercise in general, the increase in S_i with RT was significantly different from the relative decline in S_i observed in controls ($p=0.027$). At baseline, premeal FFA concentrations were lower in men than women ($\sim 30\%$, $p=0.011$), and in the younger than the elderly ($\sim 16\%$, $p=0.024$). In contrast, FFA concentrations during the last hour following meal were higher in men than women ($\sim 20\%$, $p=0.042$), while they remained lower in the young than the elderly ($\sim 35\%$, $p=0.006$). Exercise training did not significantly affect premeal FFA concentrations nor suppression by meal-induced insulin secretion. There were no statistical differences in mitochondrial O_2 flux between men and women. However, the young had higher complex I and I+II O_2 flux rates than the elderly ($\sim 24\%$, $p=0.010$; $\sim 16\%$, $p=0.050$, respectively). Moreover, both ET and CT significantly increased complex I O_2 flux rates ($\sim 28\%$, $p=0.029$; $\sim 60\%$, $p<0.001$, respectively), complex I+II O_2 flux rates ($\sim 20\%$, $p=0.010$; $\sim 50\%$, $p<0.001$, respectively) and complex II O_2 flux rates ($\sim 20\%$, $p=0.021$; $\sim 30\%$, $p<0.001$, respectively) independent of age ($p>0.05$) and sex ($p>0.05$). No association between pre- to post training changes in S_i and changes in O_2 flux was observed.

Conclusion: In summary, beta cell responsivity in response to a mixed-meal was higher in men than women and higher in the young than the elderly. Resistance exercise positively affected insulin sensitivity in the absence of improvements in mitochondrial oxidative capacity. Both endurance and combined training increased mitochondrial oxidative capacity in the absence of improvements in insulin sensitivity. In conclusion, exercise related improvements in mitochondrial function were unrelated to changes in insulin sensitivity.

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OP 43 DPP-4 inhibitors

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Blocking GLP-1 action with exendin [9-39] to determine the contribution of GLP-1 to the insulinotropic effects of the DPP-4 inhibitor vildagliptin

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Background and aims: DPP-4 inhibitors are thought to augment glucose-induced insulin secretion and suppress glucagon release by preserving higher plasma concentrations of endogenously secreted GLP-1. This view, however, has been challenged. The present study aimed at quantifying the contribution of GLP-1 to therapeutic effects of vildagliptin by using the GLP-1 receptor antagonist exendin [9-39] to specifically block GLP-1-mediated mechanisms, both in type 2 diabetic patients and in healthy subjects.

Materials and methods: 34 patients with type 2 diabetes (31m/3f; age 61 ± 9 years, BMI 28.6 ± 3.4 kg/m²; HbA_{1c} > 6.0 %; fasting plasma glucose 90–200 mg/dl; 32 completers analysed) and 34 age- and weight-matched healthy subjects (29m/5f; age 60 ± 9 years; BMI 27.0 ± 2.8 kg/m²; 30 completers analysed) participated. They were treated, in randomized order, with vildagliptin 50 mg b.i.d. or matching placebo for 10 days. Meal tests were performed on days 9 and 10, without and with the intravenous infusion of exendin [9-39] (500 pmol/kg⁻¹·min⁻¹ from -60 to 0 min followed by 350 pmol/kg⁻¹·min⁻¹ for 300 min). At -30 min, study medication was administered, and at 0 min, mixed meal ingestion was started. Glucose, insulin, C-peptide, insulin secretion rates (deconvolution), glucagon, total and intact GLP-1 and GIP were measured. The main study endpoint was the ratio of integrated incremental (AUCi) insulin secretion rates (ISR, 0–4 h) and AUCi glucose. Statistics: Friedman test (non-parametric analysis due to non-normally distributed data). Medians and inter-quartile ranges (IQR).

Results: In type 2-diabetic patients, vildagliptin treatment increased the median AUCi intact GLP-1 after meal stimulation from 2.4 (IQR 0.5;4.9) to 5.0 (1.8;9.3) pmol·h⁻¹ (p = 0.0003). Similar differences were observed for intact GIP. AUCi ISR/glucose ratios were significantly affected by treatment (p = 0.0002). Exendin [9-39] reduced the median of this ratio both when given in addition to placebo (1.5 [1.2;1.9] vs. 2.3 [1.4; 3.6] pmol/kg⁻¹·mmol⁻¹·min⁻¹; p = 0.0079) and to vildagliptin (2.0 [1.3; 2.5] vs. 2.8 [1.8; 3.8] pmol/kg⁻¹·mmol⁻¹·min⁻¹; p = 0.0039), in the latter case not, however, to the levels after placebo plus exendin [9-39] (p = 0.0028). C-peptide/glucose ratios followed a similar pattern. In healthy subjects, incretin hormones were changed similarly, but AUCi ISR/glucose or C-peptide/glucose ratios were not affected significantly. Glucagon concentrations could not meaningfully be analysed due to significant cross-reactivity of exendin [9-39] in the assay.

Conclusion: Therapeutic effects of the DPP-4 inhibitor vildagliptin on meal-stimulated insulin secretion could partially, but not completely be blocked by a GLP-1 receptor agonist administered at a dose that completely blocks insulinotropic effects of exogenous GLP-1. Therefore, some, but not all effects of DPP-4 inhibitors appear to be mediated through GLP-1. In healthy subjects, vildagliptin only affected incretin plasma concentrations, without a significant effect on insulin secretion.

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Long-term safety and efficacy of the DPP-4 inhibitor linagliptin: data from a large 2-year study in subjects with type 2 diabetes mellitus

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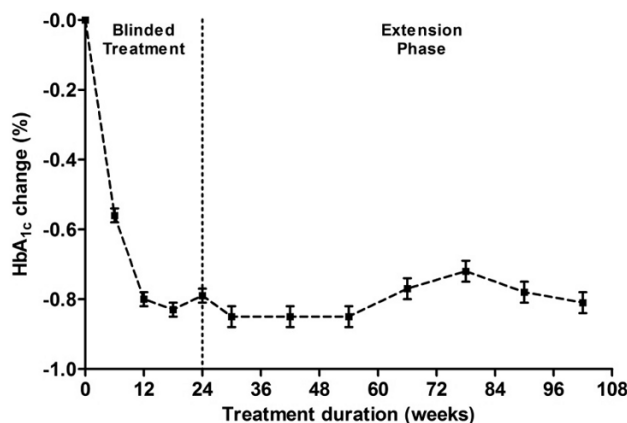
Background and aims: Linagliptin is a novel, once daily, oral DPP-4 inhibitor currently in late-stage clinical development for the treatment of type 2 diabetes mellitus (T2DM). This trial investigated the safety, tolerability, and efficacy of linagliptin in a large study population during long-term treatment.

Materials and methods: T2DM subjects who completed one of four 24-wk double-blind, placebo-controlled Phase III studies exploring linagliptin efficacy and safety as mono-, dual-, or triple therapy, could enrol in a 78-wk open-label extension study (a combined total of 2 yrs' follow up). Subjects who received placebo in 24-wk trials switched to linagliptin in the extension study. Thus, during the extension all subjects received once-daily linagliptin 5 mg as monotherapy, as combination with metformin or pioglitazone or with metformin plus sulphonylurea. Efficacy analyses were based on completers of the full analysis set (FAS) after 102 wks.

Results: In total, 2121 subjects (mean age, 58 yrs; 48.2% female; mean BMI, 29.0 kg/m²) who completed the double-blind studies after 24 wks entered the extension trial and 1826 completed 102 wks. Baseline characteristics at the beginning of treatment for the FAS completers set were balanced between those randomized to linagliptin (n = 1310; HbA_{1c}, 8.1%) or placebo (n = 516; HbA_{1c}, 8.2%). Significant reductions in HbA_{1c} achieved with linagliptin after 24 wks' blinded treatment (-0.8%) were sustained over the further 78-wks extension (2-yr change vs. baseline of -0.8%; Figure). For those initially randomized to placebo, switching to linagliptin at wk 24 resulted in an additional decrease in HbA_{1c} of -0.5% after the extension period. Treatment with linagliptin continued to be well tolerated with no major trends of clinical relevance during the extension study (up to 2 yrs). The overall incidence of hypoglycaemic events during the extension period was similar for subjects continuing with linagliptin (14.6%) and those switching from placebo (13.6%). Approximately one-third of subjects in each group were receiving background metformin plus sulphonylurea therapy, hypoglycaemic events were most frequent in these subjects. Body weight was not substantially altered after switching to linagliptin or continuing linagliptin during long-term treatment.

Conclusion: Linagliptin provides sustained long-term glycaemic control when used as monotherapy or add-on to other oral glucose-lowering agents. Linagliptin treatment was well tolerated without clinically relevant increases in the risk of hypoglycaemia or causing weight gain.

HbA_{1c} (%) mean change over time for patients who received linagliptin at the start of the 2-year treatment period



Patient numbers: wk 6, n=1279; wk 12 n=1258; wk 18, n=1241; wk 24, n=1226; wk 30, n=1213; wk 42, n=1174; wk 54, n=1097; wk 66, n=1025; wk 78, n=958; wk 90, n=910; wk 102, n=903

Clinical Trial Registration Number: NCT00736099

Supported by: Boehringer Ingelheim

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Saxagliptin add-on therapy to insulin with or without metformin for type 2 diabetes mellitus: 52-week safety and efficacy

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Background and aims: The safety and efficacy of the dipeptidyl peptidase-4 inhibitor saxagliptin (SAXA) were evaluated as add-on therapy in patients with type 2 diabetes mellitus (T2DM) inadequately controlled with insulin (INS) alone or INS combined with metformin (MET).

Materials and methods: In a phase IIIB, double-blind, parallel-group trial, adults with HbA_{1c} 7.5–11% on stable INS (30–150 U/d ± MET) for ≥8 weeks

at screening were stratified by MET use and randomly assigned 2:1 to oral SAXA 5 mg/d (n=304) or placebo (PBO; n=151) for a 24-week short-term (ST) period followed by a 28-week long-term (LT) period (52 weeks total). MET doses were kept stable. INS doses were kept stable during the ST period before rescue, but could be adjusted without rescue during the LT period to optimize glycemic control.

Results: Mean age was 57 y, mean T2DM duration was 12 y, and mean baseline (BL) HbA_{1c} was 8.7%. LT completion rates were similar in the SAXA (81%) and PBO groups (83%). Most patients used MET along with INS (69% in the SAXA arm [mean BL dose, 1805 mg/d]; 70% in the PBO arm [mean BL dose, 1861 mg/d]). The most common INS regimens were premixed (57% of patients), intermediate acting (19%), and long acting (18%). In repeated measures analysis, adjusted mean HbA_{1c} decreased more from BL to week 52 with SAXA (−0.8%) vs PBO (−0.4%; difference, −0.4% [95% CI: −0.6 to −0.2]), whether MET was used (difference, −0.4% [95% CI, −0.6 to −0.2]) or not (difference, −0.4% [95% CI, −0.7 to −0.03]). At week 52, 21.3% of SAXA-treated patients and 8.7% of patients receiving PBO achieved HbA_{1c} <7% (difference, 12.6% [95% CI, 6.1 to 19.1]). More SAXA-treated patients vs patients in the PBO group achieved HbA_{1c} <7%, whether MET was used (23.8% vs 8.7%) or not (16.0% vs 8.7%). Mean total daily INS dose increased from BL in both treatment groups through week 52, with a numerically smaller increase in the SAXA group (repeated measures, 5.7 vs 6.6 U; 95% CI for difference, −3.2 to 1.3). SAXA + INS was well tolerated vs PBO + INS: 66.4% and 71.5% of patients had ≥1 adverse event (AE) and few patients discontinued owing to AEs in the SAXA and PBO groups (3.0% and 2.0%, respectively). Overall AE incidences were similar regardless of MET use. Most AEs were mild or moderate. Serious AEs were infrequent and balanced across groups. Two deaths in SAXA-treated patients (myocardial infarction, intestinal necrosis) were judged unrelated to study drug. The most common AE classes were infections and infestations (35.5% vs 41.1%, SAXA vs PBO), gastrointestinal disorders (18.8% vs 16.6%), and musculoskeletal and connective tissue disorders (16.4% vs 19.9%). Patients in the SAXA and PBO groups both experienced small, similar weight gains (adjusted mean change from BL [repeated measures], 0.8 vs 0.5 kg; 95% CI for difference, −0.3 to 0.8). Hypoglycemia was reported in 22.7% of SAXA-treated patients and 26.5% of patients receiving PBO. Hypoglycemia was confirmed (fingerstick glucose ≤50 mg/dL [2.8 mmol/L] with associated symptoms) in 7.6% of SAXA-treated patients and 6.6% of patients in the PBO group.

Conclusion: SAXA 5 mg add-on therapy improved glycemic control in patients with T2DM on INS alone or INS + MET, an effect sustained through 52 weeks. Despite the flexible INS regimen, SAXA did not increase risk of hypoglycemia and was well tolerated.

Clinical Trial Registration Number: NCT00757588

Supported by: BMS and AZ

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Dipeptidyl peptidase-4 inhibitors and cardiovascular events: a protective effect?

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Background and aims: Data from randomized clinical trials with metabolic outcomes can be used to address concerns about potential issues of cardiovascular safety for newer drugs for type 2 diabetes. This meta-analysis was designed to assess cardiovascular safety of Dipeptidyl Peptidase-4 inhibitors (DPP-4i).

Materials and methods: Medline, Embase, Cochrane databases, and www.clinicaltrials.gov website were searched for randomized trials of DPP-4 inhibitors (versus placebo or other comparators) with duration ≥ 24 weeks, performed in type 2 diabetic patients. Mantel-Haenszel odds ratio with 95% Confidence Interval (MH-OR) was calculated for major cardiovascular events (MACE), on an intention-to-treat basis, excluding trials with zero events.

Results: Fifty-three trials enrolling 20,312 and 13,569 patients for DPP4i and comparators, respectively, were included, reporting 257 MACE. DPP4i, compared with placebo or other treatment, were associated with a reduced risk of MACE (MH-OR 0.689 [0.528;0.899], p=0.006).

Conclusion: The present meta-analysis suggests a possible protection from cardiovascular events with the use of DPP-4i.

OP 44 Nonalcoholic fatty liver disease

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Glucagon-like peptide-1 receptor (GLP-1R) agonism ameliorates non-alcoholic steatohepatitis (NASH) in rodent models

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Background and aims: Pharmacotherapies for non-alcoholic steatohepatitis (NASH) are lacking. The present studies aimed to expand on the potential beneficial effect of GLP-1 receptor agonism in rodent models of NASH.

Materials and methods: We examined the effects of the exenatide analog AC3174 (30 µg/kg/d for 28 days) in Lep^{ob}/Lep^{ob} and C57BL6J (B6) mice that were maintained on a high trans-fat/high fructose/high cholesterol (HTF) diet. In a separate study, the effects of AC3174 were compared to (drug-naïve) calorie-restricted weight matched controls. AC3174 was also administered to GLP-1 receptor-deficient (Glp1rKO) mice.

Results: The HTF diet increased micro- and macro-steatosis in both strains of mice. In Lep^{ob}/Lep^{ob}, but not B6 mice, induction of fibrosis coupled with a ~6-fold increase in collagen-1 mRNA and protein was also evident. AC3174 treatment reduced: body weight (by 7.3% in Lep^{ob}/Lep^{ob} and 15.5% in B6 mice vs vehicle), liver mass (by 20% and 30%), liver lipid (by 11% and 38%), and attenuated fibrosis. In the next study, AC3174-treated mice exhibited significantly reduced body weight (8.3%), liver mass (14.2%), liver lipid (12.9%), plasma alanine aminotransferase, and triglycerides, whereas a calorie-restricted weight-matched group demonstrated only modest non-significant reductions in liver mass (9%) and liver lipid (5.1%) relative to controls. Treatment of Glp1rKO mice with AC3174 had no effect on body weight, adiposity, hepatic, or plasma indices, suggesting that the observed beneficial effects of AC3174 are mediated via the known GLP-1 receptor.

Conclusion: Together, these data support the clinical evaluation of the utility of GLP-1 receptor agonists for treatment of NASH.

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Non alcoholic fatty liver disease (NAFLD) is a major risk factor for cardiovascular disease in patients with type 2 diabetes mellitus

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Introduction: Non-alcoholic fatty liver disease is a spectrum of metabolic liver disease that extends from: bland steatosis (fatty liver), where the histology and the function of the liver are not affected, and non alcoholic steatohepatitis, where steatosis, pericellular and hepatocellular inflammation, Mallory bodies, fibrosis and elevation of liver biochemical parameters (AST, ALT) are present to cirrhosis and hepatocellular cancer as a result of NAFLD. Aim: To determine if NAFLD is a major risk factor for cardiovascular disease in patients with type 2 diabetes mellitus.

Materials and methods: The study included 468 patients with type 2 DM, out of them 290 were men (62%) and 178 (38%) were women, with mean age of 62±13 years old. All the patients underwent liver ultrasound scan and biochemical exams (fasting glucose, HbA_{1c}, AST, ALT, γ-GT, total bilirubin) during 3 years.

Results: The patients were divided in 2 groups: group A consisted of 184 (39%) patients with NAFLD and DM and group B consisted of 284 (61%) patients with type 2 DM without NAFLD. During the 3 years mean follow up a major cardiovascular event (non fatal myocardial infarction, revascularisation or cardiovascular death) occurred in 102 (55%) patients of group A and only in 42 (15%) of group B.

Conclusion: NAFLD is a major risk factor for cardiovascular events in Patients with type 2 diabetes mellitus. These patients should be treated and often examined both by an internist and a cardiologist. Early start of treatment for diabetes mellitus and strict control of blood sugar prevent cardiovascular events and the progress of liver disease.

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Non-alcoholic fatty liver disease severity is related to increased cardiovascular risk independently of hyperglycaemia and obesity

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a common chronic condition which is strongly linked to obesity and diabetes, being prevalent in up to 90% of obese subjects and up to 70% of people with type 2 diabetes. The risk of cardiovascular (CV) disease is significantly increased in NAFLD and represents the main cause of death in these patients. NAFLD forms a spectrum of fat-related liver conditions extending from simple and relatively harmless steatosis, to end-stage progressive liver disease requiring transplantation because of decompensated cirrhosis or hepatocellular carcinoma. Non-invasive techniques are available to quantify the severity of fat infiltration and to assess fibrosis. However, histopathological assessment of liver tissue remains the gold standard to assess NAFLD severity. Although NAFLD is associated with obesity and diabetes, the precise nature of the relationship between NAFLD severity, hyperglycaemia, obesity and CV risk is uncertain. Our aim was to establish the relationships between a histopathological marker of NAFLD disease severity, levels of glycaemic control, obesity and a 10-year CV risk estimate.

Materials and methods: Retrospective data on patients with biopsy-proven NAFLD were obtained using patient hospital notes and the electronic patient management system 'e-Quest'. These data were utilized to calculate CV risk using online QRISK2 and Framingham heart study 10-year risk calculators (three patients were omitted due to documented CV disease). NAFLD severity was assessed by histopathological evaluation of liver biopsy specimens using the 12-point Kleiner scoring system. Total and truncal fat were measured using dual energy x-ray absorptiometry (DEXA) scans.

Results: NAFLD severity by Kleiner score was available on $n=104$ people (67 men). Kleiner score was 5.3 ± 2.3 (mean \pm SD), (range 1–11). 32 subjects had a prior diagnosis of diabetes. Kleiner score in people with diabetes, compared to without, was 6.4 ± 2.0 and 4.8 ± 2.1 respectively ($p < 0.001$). The age of the whole cohort was 47.9 ± 12.6 years (mean \pm SD). Kleiner score was associated with fasting glucose ($r=0.21$, $p=0.038$), HbA_{1c} ($r=0.26$, $p=0.034$) and 10-year CV risk calculated by both QRISK2 ($r=0.458$, $p < 0.001$) and Framingham risk score (FRS) ($r=0.374$, $p < 0.001$). There was no relationship between BMI and Kleiner score ($r=0.06$, $p=0.58$), although in a sub-group ($n=45$), there was a trend toward an association between Kleiner score and truncal fat percentage ($r=-0.29$, $p=0.077$). In multivariable linear regression analyses that included measures of glycaemic control (glucose and HbA_{1c}) and BMI, both QRISK2 (β -coefficient 0.42, $p=0.001$) and FRS (β -coefficient 0.38, $p=0.003$) were strongly and independently associated with Kleiner score.

Conclusion: These data show for the first time a strong association between a histological measure of NAFLD severity and calculated estimates of CV risk, which was independent of markers of glucose control and obesity.

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Prevalence and markers of advanced liver disease in people with type 2 diabetes: the Edinburgh type 2 diabetes study

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Background and aims: Type 2 diabetes is a risk factor for the progression of hepatic steatosis and non-alcoholic fatty liver disease (NAFLD) to fibrosis and cirrhosis. We examined the prevalence of hepatic fibrosis and portal hypertension, in addition to hepatocellular carcinoma (HCC), in people with type 2 diabetes, using the non-invasive markers hyaluronic acid (HA) and platelet count/spleen diameter ratio (PSR), and analysed the effectiveness of liver function tests (LFTs) in screening for liver disease in this population.

Materials and methods: Subjects recorded as having type 2 diabetes, aged 61–76 years, were invited at random from the Lothian Diabetes Register to participate in the Edinburgh Type 2 Diabetes Study. Ultrasonography was performed in 939 participants to record cirrhosis, spleen size (mm) and focal liver abnormalities. Serum HA (ng/ml), platelets (/mm³), alpha fetoprotein (AFP, kU/l), bilirubin (Bi, umol/l), alanine transferase (ALT, units/l) and gamma glutamyltransferase (GGT, units/l) were measured. Participants with no secondary cause for liver disease (possible NAFLD) were split into 5 groups according to increasing levels of fibrosis (Group 1, normal; Group 2, hepatic steatosis alone; Group 3, HA 50–100 ng/ml, no arthritis; Group 4, HA 100–300 ng/ml, no arthritis; Group 5, cirrhosis, PSR < 909 or HA > 300 ng/ml, no arthritis).

Results: Cirrhosis was identified by ultrasound in 4 participants (0.4%). 10 (1.1%) had evidence of portal hypertension (PSR < 909). 222 (23.6%) had evidence of hepatic fibrosis (HA > 50 ng/ml, no arthritis), and 53 of these had HA > 100 ng/ml. Of 663 participants with no secondary cause for liver disease, 1 (0.2%) had cirrhosis, 4 (0.6%) had PSR < 909 and 166 (25.0%) had HA > 50 ng/ml of whom 40 had HA > 100 ng/ml. Two participants had HCC, a prevalence in the study population of 0.2%. Tests of liver function were compared across the Fibrosis Groups (Table 1). Bi, ALT and GGT were highest in Groups 4 and 5, with statistically significant differences between groups ($p < 0.05$). Mean levels remained within normal limits in all groups. The positive predictive values of ALT and GGT levels above normal in predicting fibrosis (i.e. Groups 3–5) were 26.1% and 28.6%. The negative predictive values of normal values were 75.2% and 75.3% respectively.

Conclusion: The prevalence of hepatic fibrosis was high, but that of cirrhosis or likely portal hypertension was lower than anticipated. The use of conventional LFTs to screen for liver disease missed a significant proportion of cases of fibrosis predicted by raised HA levels.

Table 1

	Group 1 (n=197)	Group 2 (n=294)	Group 3 (n=120)	Group 4 (n=36)	Group 5 (n=8)
Bi (umol/l)	8.6 \pm 4.6	8.0 \pm 3.5	10.0 \pm 7.6	9.8 \pm 4.3	12.0 \pm 4.4 [#]
Bi > 18 (no. (%))	9 (4.6)	5 (1.7)	8 (6.7)	2 (5.6)	1 (12.5)
ALT (U/l)	30.1 \pm 9.8	35.5 \pm 13.7	33.8 \pm 12.8	34.5 \pm 14.6	37.6 \pm 10.4 [#]
ALT > 50 (no. (%))	5 (2.5)	29 (9.9)	7 (5.8)	4 (11.1)	1 (12.5) [#]
AST (U/l)	28.6 \pm 7.9	30.9 \pm 10.8	31.0 \pm 8.8	35.1 \pm 12.9	40.4 \pm 14.4 [#]
AST > 45 (no. (%))	6 (3.0)	21 (7.1)	6 (5.0)	4 (11.1)	2 (25) [#]
GGT (logGGT)	1.1 \pm 0.3	1.3 \pm 0.3	1.2 \pm 0.3	1.4 \pm 0.5	1.5 \pm 0.5 [#]
GGT > 55 (no. (%))	11 (5.6)	24 (8.2)	6 (5.0)	6 (16.7)	2 (25.0) [#]

* $p < 0.05$ on one-way ANOVA or chi-squared across the 5 groups.

Bi, bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT gamma glutamyltransferase. Data are mean \pm standard deviation unless otherwise stated

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OP 45 Hypoglycaemia

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High levels of stimulated glucagon 12 months after diagnosis of type 1 diabetes decrease the risk of severe hypoglycaemia in the following years

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Background and aims: The risk of severe hypoglycaemia (SH) is the major factor for preventing good glycemic regulation as assessed by HbA_{1c}. The aim of the study is identification of individuals at high risk for acute diabetic complications within one year after onset of disease.

Materials and methods: A sample of 129 children and adolescents < 17 years from four centres in Denmark with newly diagnosed Type 1 Diabetes (T1D) diagnosed from April 2004 to April 2005 were followed for 6 years, prospectively. HbA_{1c}, liquid mixed-meal stimulated C-peptide and glucagon were analyzed centrally 1, 3, 6 and 12 months after diagnosis. Data such as gender, age and events of SH, including annual recordings of HbA_{1c} were recorded. SH was defined as unconsciousness and/or seizures and a blood glucose level < 3.5 mmol/L. Logistic regression was used to analyse the influence of glucagon and C-peptide levels after 12 months on the subsequent risk of severe hypoglycaemic events in the following 6 years.

Results: 129 children were included, 66 boys (51 %) and 63 girls (49 %). 94 children did not experience SH (mean age 10.3 (range 1.5 - 16.7) years), and 30 children had a severe hypoglycaemic event (mean age 9.0 (range 0.6 - 15.7) years), (missing data for age on 5 children). There were 7 children who experienced a severe hypoglycaemic event within the first year after diagnosis and 30 children experienced SH over the following 6 years. Age at onset and gender was not associated with an increased risk of SH. Individuals with severe hypoglycaemic events within the first year had a 15 times risk of future severe hypoglycaemic events ($p < 0.01$). Median glucagon levels at 12 months among 30 children who experienced SH over the six years follow-up was 7.0 (range 3.0 - 15.0) pmol/L. Median stimulated C-peptide levels at 12 months in the same group was 236.0 (range 10.0 - 1096.0) pmol/L. There is a 19.3% decreased risk of long-term SH (95% CI 4.5-31.6%) per [pmol/L] increase of glucagon at 12 months, after adjusting for C-peptide levels and first-year severe hypoglycaemic events in the statistical analyses.

Conclusion: Early severe hypoglycaemic events and decreased stimulated glucagon levels at 12 months seem to identify individuals at higher risk of future severe hypoglycaemic events indicating a role for glucagon in preventing SH in some individuals. How glucagon should be incorporated in future treatment of diabetes needs further investigations.

Supported by: Herlev University Hospital

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Quantitative EEG in type 1 diabetic adults with childhood exposure to severe hypoglycaemia: a 16-year follow-up study

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Background and aims: In diabetic children, a history of severe hypoglycaemia (SH) has been associated with increased slow EEG activity. Our aim was to assess whether EEG abnormalities associated with childhood SH persist into adulthood.

Materials and methods: In 1992, we studied quantitative EEG in 28 diabetic children and 28 matched controls. Sixteen years later, we repeated the investigations in 96% of this cohort. Diabetic subjects were classified as with ($n = 9$) or without ($n = 18$) early SH, defined as episodes with convulsions or loss of consciousness by ten years of age. For each EEG band (delta, theta, alpha, and beta) and cerebral region (frontocentral, temporal, and parietoccipital), we calculated relative amplitudes and amplitude asymmetry. We used a mixed linear model to calculate occipital alpha mean frequency, alpha peak frequency at maximum amplitude and alpha peak width. We examined whether these EEG measures, relative to age- and sex-matched controls, differed between diabetic subjects with and without early exposure to SH. In separate analyses, we adjusted for blood glucose at the EEG recording.

Results: Participants were 27 diabetic (13 female) and 27 control subjects (13 female), age (mean(SD) 28.1(2.2)/ 28.8(2.0) / 28.7(2.1) years (diabetic subjects with /without early SH/ controls). In diabetic subjects with and without early SH, age at diabetes onset was 5.4 (2.6) and 9.8 (2.9) years, diabetes duration 22.7 (2.8) and 19.1 (1.8) years, lifetime number of SH episodes (median (range)) 7 (3-27) and 3 (0-14), mean HbA_{1c} since diabetes onset (weighted for frequency of measurements) 9.3 (1.1) and 8.5 (0.7) %, number of HbA_{1c} measurements 72 (12) and 53(13), and blood glucose at EEG recording (median (range)) 11.6 (5.5-23.3) and 10.1 (4.8-21.0) mmol/l, respectively. We found no association between early exposure to SH and quantitative EEG variables neither in the frontocentral region (Table) nor in any other cerebral region. The total number of SHs and mean HbA_{1c} levels were not significantly associated with regional mean relative amplitudes or EEG frequencies, and adjustment for blood glucose at the recordings did not substantially influence the results (data not shown).

Conclusion: Early exposure to SH was not associated with EEG abnormalities in young adults with type 1 diabetes. Our results suggest that EEG abnormalities associated with childhood exposure to SH do not persist into adulthood. Table: Mean relative EEG amplitudes in the frontocentral region in diabetic subjects and controls, by early^a exposure to SH, and the association of early SH with mean relative amplitudes.

Mean relative EEG amplitudes (%)							
	Diabetes with early SH		Diabetes without early SH		Association with early SH		
	Diabetic subjects	Controls	Diabetic subjects	Controls	Difference ^b	(95% CI)	P ^c
Delta	17.1	18.1	18.9	20.6	0.7	(-3.4 - 4.8)	0.73
Theta	16.9	16.6	17.7	18.5	1.1	(-2.9 - 5.2)	0.57
Alpha	26.8	28.3	25.6	24.1	-3.0	(-10.0 - 4.0)	0.39
Beta	39.2	37.0	37.7	36.8	1.2	(-6.4 - 8.8)	0.76

^aby 10 years of age

^bdifference in relative amplitude (in percentage points) associated with early SH

^cP for interaction between having diabetes and being part of a diabetes-control pair in which the diabetic patient had early SH

Clinical Trial Registration Number: 2406

Supported by: NTNU; Trondheim University Hospital; Norwegian Diabetes Association; Unimed

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The relationship between plasma and brain glucose levels under hypoglycaemic conditions in humans

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Background and aims: Since glucose is the main source of energy for the brain, understanding the kinetics of brain glucose uptake under hypoglycaemic conditions is highly important. Under normo- and hyperglycaemic conditions the relationship between plasma and brain glucose levels is linear and behaves according to reversible Michaelis-Menten (MM) kinetics. The present study was conducted to test whether these kinetics also describe the relationship between plasma and brain glucose during hypoglycaemia, by measuring human cerebral glucose content during hypoglycaemia using ¹³C magnetic resonance spectroscopy (MRS).

Materials and methods: Eight healthy volunteers underwent ¹³C MRS at a magnetic field strength of 3T in a 125 ml voxel in occipital brain tissue during 2-hour hyperinsulinemic (60 mU/min/m²) euglycaemic and hypoglycaemic glucose clamps, performed in random order on separate days. [1-¹³C]glucose was infused during the clamps to increase plasma ¹³C isotopic enrichment. Plasma glucose and plasma ¹³C enrichment were measured every 5 minutes. MR spectra acquired during steady-state ($t = 50$ -100 min) were averaged. The peaks of α - and β -glucose were fitted with the AMARES algorithm in jMRUI. To quantify metabolite levels the natural abundance ¹³C myo-inositol signals were assumed to represent 1.1% of 6 mM. In addition, glucose signals were corrected for the ¹³C enrichment of plasma glucose and for 5% contamination by blood vessels. The values of reversible MM kinetic parameters (T_{max}/CMR_{glc} and K_1) for glucose transport were calculated from plasma and brain glucose values during steady-state.

Results: Plasma glucose levels ranged from 4.38-5.26 (normoglycaemic) to 2.54-3.26 mM (hypoglycaemic) during steady-state. The quality of the ¹³C-

MR spectra was good under both conditions. Steady-state brain glucose levels averaged $0.5 \pm 0.2 \mu\text{mol/g}$ during hypoglycaemia (plasma glucose, $3.0 \pm 0.3 \text{ mM}$) and $1.3 \pm 0.5 \mu\text{mol/g}$ during euglycaemia (plasma glucose, $5.1 \pm 0.3 \text{ mM}$). Individual steady-state brain glucose levels as a function of plasma glucose concentrations are presented in figure 1. The best fit of the data to the reversible MM kinetic model resulted in the following values for the glucose transport parameters: $T_{\text{max}}/\text{CMR}_{\text{glc}} = 2.93 (-)$ and $K_t = 3.27 \text{ mM}$.

Conclusion: This study demonstrates that the relationship between steady-state plasma and brain glucose under hypoglycaemic conditions in humans is linear and follows reversible MM kinetics. These data extend those previously obtained under normo- and hyperglycaemic conditions, and provide further refinement of the reversible MM kinetic parameters in humans *in vivo*.

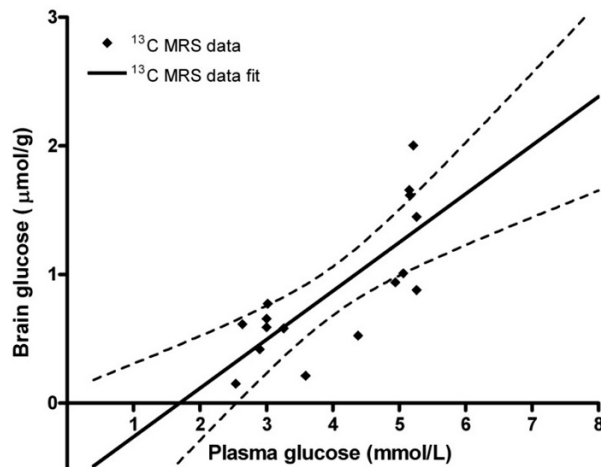


Figure 1: Plasma versus brain glucose relationship as measured by ^{13}C MRS (diamonds) together with linear regression fit (solid line) and 95% confidence intervals (dashed lines).

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Relationship of hypoglycaemia with QTc prolongation in patients with type 2 diabetes

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Background and aims: Recent clinical studies show that hypoglycemia is associated with increased risk of death, mainly attributed to lethal arrhythmias and QTc prolongation. This is mostly studied in type 1 diabetes patients. The aim of the present study was to study the relationship of hypoglycemic episodes (examined during continuous glucose monitoring subcutaneously [CGMS]) with QTc prolongation (measured by continuous ECG monitoring) in patients with type 2 diabetes (T2D).

Materials and methods: A total of 26 (14 males) patients with T2D (mean age \pm SD): 60.3 ± 10.9 years, HbA_{1c} : $6.73 \pm 0.73\%$, diabetes duration 6.9 ± 5.0 years, 7 treated with insulin, 19 treated with insulin secretagogues) were studied with simultaneous CGMS and 24-hour ECG monitoring. Hypoglycemia was defined as episodes of blood glucose (BG) $<70 \text{ mg/dl}$ (3.9 mmol/L), lasting for >5 minutes, while hyperglycemia as episodes of BG $>200 \text{ mg/dl}$ (11.1 mmol/L), lasting for >5 minutes. The mean QTc of these episodes was compared with the mean QTc of normoglycemic intervals (BG between 70 – 120 mg/dl [3.9 – 6.7 mmol/L] of similar duration).

Results: A total of 26 non-severe hypoglycemic episodes in 5 insulin-treated and 8 insulin secretagogue-treated patients were identified. Mean BG during hypoglycemia was 59.2 [95% CI 56.4 – 62.0] mg/dl , while BG during normoglycemia was 98.7 [90.4–107.0] mg/dl . Mean QTc during hypoglycemic episodes was significantly higher than during normoglycaemia (431.4 [416.6–446.2] vs. 416.6 [397.6–435.5] msec, $p=0.015$).

Conclusion: Mild/moderate hypoglycemia in patients with T2D is associated with prolongation of the QTc interval and may contribute to the increased adverse outcomes seen with intensive treatment of the disease.

OP 46 Islet electrophysiology

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The Cav1.2 calcium channel spatial expression in pancreatic beta cells is regulated by eIF3e - eukaryotic translation initiation factor subunit 'e'

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Background and aims: Cav1.2 channels regulate insulin secretion in rodent beta-cells. The magnitude of cytosolic calcium may be controlled via changing calcium channel density in the plasma membrane by altering the balance between channels trafficking to and from the cell surface. This process is poorly elucidated, but it is known that beta and alpha2delta auxiliary subunits of L-type calcium channels are implicated in Cav1.2 surface expression whilst little is known about other regulators. In this study we investigated whether the eukaryotic translation initiation factor subunit 'e' (eIF3e) acts as a regulator of Cav1.2 surface expression in beta-cells.

Materials and methods: The interaction between Cav1.2 and eIF3e was investigated by immunoprecipitation and confocal microscopy in INS-1 cells. Cav1.2 distribution was assessed by immunostaining and quantitative comparison of the average signal intensity of Cav1.2 in the plasma membrane and the cytoplasm. The role of eIF3e in Cav1.2 surface expression was evaluated by using RNAi silencing in Cav1.2-GFP transfected INS-1 cells and subsequent real-time imaging. Apoptosis was measured as % of annexin-V positive cells in siRNA treated compared to untreated INS-1 cells at low (5 mM) and high (20 mM) glucose concentrations.

Results: INS-1 cells stimulated with high glucose revealed a substantial shift of Cav1.2 localization from the plasma membrane (PM) to the cytoplasm (CP). Glucose decreased the PM/CP expression ratio in immunostained cells from 1.40 ± 0.09 before, to 0.83 ± 0.05 after 30-min glucose stimulation. Similarly, in real-time imaging experiments the PM/CP ratio of Cav1.2-GFP fluorescence decreased from 1.22 ± 0.04 to 0.49 ± 0.06 after glucose stimulation. The eIF3e PM/CP expression ratio was also determined by immunostaining and amounted 1.11 ± 0.03 and 0.74 ± 0.10 in low and high glucose, respectively. The eIF3e interaction with Cav1.2 was revealed by immunoprecipitation. This interaction was glucose dependent as shown by confocal immunocytochemistry. After incubation at low glucose (30 min), Cav1.2 and eIF3e co-localization was $95 \pm 5\%$, whereas after 30 min in high glucose, it was reduced to $41 \pm 4\%$. Quantitative real-time imaging revealed that in eIF3e silenced INS-1 cells, the PM/CP expression ratio of Cav1.2-GFP decreased from 0.98 ± 0.07 to 0.91 ± 0.09 in INS-1 by glucose stimulation. The eIF3e protein exhibited glucose dependent effects on viability in both INS-1 cells and human pancreatic islets. In INS-1 cells cultured in low glucose apoptotic events were seen in $3.1 \pm 0.1\%$ of the cells, which increased slightly to $3.4 \pm 0.1\%$ in cells cultured for 6 h in high glucose. Knock-down of eIF3e increased apoptosis to $5.2 \pm 0.1\%$ in low glucose, and this effect was massively stimulated in high glucose ($28.6 \pm 0.3\%$ apoptotic cells). Finally, glucose-induced apoptosis was investigated in human islets, and amounted $3.3 \pm 0.8\%$ and $22.3 \pm 1.2\%$ after 72 h culture at 5 and 20 mM glucose, respectively. Silencing of eIF3e did not change apoptosis ($3.2 \pm 0.5\%$) at low glucose, but in 20 mM glucose it was doubled to ($45 \pm 5\%$).

Conclusion: Our study shows that stimulation-dependent Cav1.2 internalization requires eIF3e, which directly interacts with Cav1.2. eIF3e knockdown makes both rodent and human beta-cells more vulnerable to glucose-induced apoptosis.

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Two pools of insulin granules with different dependence on Ca^{2+} entry are involved in rapid exocytosis

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Background and aims: Biphasic insulin secretion has been suggested to be caused by release of distinct pools of secretory granules. Such pools can be studied using membrane capacitance measurements and the patch-clamp technique. This study investigated how different phases of capacitance traces are shaped by pool depletion, calcium-current inactivation, and spatial organization using different depolarization protocols and mixed-effects statistical modeling.

Materials and methods: The whole-cell patch-clamp technique was used to measure depolarization-evoked capacitance increases reflecting exocytosis in

INS-1 832/13 cells. Exocytosis was analyzed as a function of Ca^{2+} entry using mixed-effects statistical models to account for cell heterogeneity.

Results: In response to depolarization of different lengths (10–640 ms), capacitance increases (ΔCm) reflecting exocytosis showed a typical biphasic pattern, where the rate of exocytosis was higher for shorter than for longer pulses. This pattern was unchanged by 7 mM free intracellular EGTA when free intracellular $[\text{Ca}^{2+}]$ was ~ 60 nM. The mixed-effects model included a fixed-effect of Ca^{2+} charge Q on ΔCm , which reports the overall Ca^{2+} sensitivity, a fixed-effect offset, and a random effect to allow for cell-to-cell variability. Ca^{2+} sensitivity was estimated to 0.82 ± 0.20 fF/pC, the offset was 5.9 ± 2.0 fF (significantly different from zero, $p=0.0034$, t -test), and cell heterogeneity was significant ($p<0.0001$, F -test). Again, high EGTA levels had no significant effect. Next, we applied a 50 ms pre-pulse 100 ms before another 50 ms pulse. The second pulse evoked significantly smaller exocytosis than the first one (5.0 ± 1.3 vs. 12.1 ± 3.6 fF, $p=0.02$, $n=11$, Wilcoxon paired signed-rank test). A linear model estimated that the Ca^{2+} sensitivity of the second pulse was significantly reduced ($p<0.002$, t -test) by $50 \pm 11\%$. Thus, a small immediately releasable pool (IRP) of granules is depleted within 100 ms. We combined the above protocols, so that each depolarization in a pulse-length protocol (50–800 ms) was preceded by a 50 ms pre-pulse. The capacitance increase of this second depolarization (ΔCm_2) as a function of duration was parallel to the response triggered by depolarizations without pre-pulses, with a downward shift comparable to the capacitance increase of the pre-pulse. In agreement, a mixed-effect analysis of ΔCm_2 as a function of Ca^{2+} entry showed a similar Ca^{2+} sensitivity of 0.83 ± 0.20 fF/pC, and cell heterogeneity was significant ($p<0.0001$, F -test), but in contrast to the results with no pre-pulse, the estimated offset-value was not significantly different from zero and amounted to -1.5 ± 3.3 fF.

Conclusion: We suggest that first-phase release reflects depletion of a small IRP and later exocytosis is due to recruitment of other granules. The latter phase is not due to refilling of the IRP, but rather because of release of another pool, such as a highly Ca^{2+} sensitive pool (HCSP) of granules. The lack of effect of high concentrations of EGTA suggests that both pools are situated within a few hundred nanometers from Ca^{2+} channels.

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Bi-directional granule turnover in the submembrane space during K^+ depolarisation-induced secretion

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Background and aims: The monophasic secretion elicited by a KCl depolarization is believed to correspond to the first phase of glucose-induced insulin secretion, in that both represent the release of a pool of membrane-adjacent secretory granules which await one final trigger, a depolarization-induced influx of Ca^{2+} . A number of recent observations have put this hypothesis into question.

Materials and methods: Insulin secretion was measured by perfusion of mouse islets and MIN6 pseudo-islets and ELISA of the fractionated effluat. The free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) of islets and MIN6 cells was measured with the Fura technique. Granules in the submembrane space were visualized by transient transfection of MIN6 cells with an insulin-EGFP fusion protein and imaging by TIRF microscopy (decay constant 80 nm) at 37°C . The images were evaluated by an in-house written program to achieve an observer-independent quantitation of the entire granule population.

Results: Like primary mouse islets, MIN6 pseudo-islets responded to the depolarization by 40 mM KCl and the resulting increase of the $[\text{Ca}^{2+}]_i$ with a massive increase in insulin secretion, whereas 15 mM KCl had little effect in spite of a clear increase of $[\text{Ca}^{2+}]_i$. Analysis of insulin-EGFP-labelled granules in MIN6 cells by TIRF microscopy showed that 40 mM KCl increased the number of short term-resident granules (less than 1 s presence in the submembrane space) while the total granule number and the number of long-term resident granules decreased. The rates of granule arrival at and departure from the submembrane space changed in parallel and were two orders of magnitude higher than the release rates, suggesting a back-and-forth movement of the granules as primary determinant of the submembrane granule number. The parameters of mobility parallel to the membrane were unchanged by the depolarization when corrected for the increased share of short term residents. The effect of 15 mM KCl resembled that of 40 mM but did not achieve significance. Both 15 and 40 mM KCl evoked a $[\text{Ca}^{2+}]_i$ increase, which was antagonized by 10 μM nifedipine. Nifedipine not only

antagonized the effect on secretion and exocytosis but also on submembrane granule number and mobility. The mobility parameters of the comparatively few granules that underwent exocytosis were remarkable in that they combined very long total itineraries with short net itineraries, suggesting intensified mobility within a cage. None of the short term resident granules was found to fuse.

Conclusion: During KCl-depolarization L-type Ca^{2+} channels seem to regulate two separate processes, granule turnover in the submembrane space and the insulin granule release. In contrast to current models of granule transport, which are strictly sequential, the majority of insulin granules present in submembrane space may return to a more distant pool without undergoing exocytosis.

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The influence of beta cell microfilaments on insulin secretion depends on the source of triggering calcium

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Background and aims: Glucose- or sulphonylurea-induced insulin secretion depends on depolarization-mediated influx of Ca^{2+} through voltage-dependent calcium channels (VDCC) in beta cells. The ensuing rise in the cytosolic concentration of ionized Ca^{2+} ($[\text{Ca}^{2+}]_i$) triggers exocytosis of insulin granules thought to be localized near the inner mouth of VDCC. Disruption of the sub-membrane web of actin microfilaments facilitates access of granules to these release sites. It is not known whether microfilaments also influence the size of granule pools that are released when triggering Ca^{2+} originates from intracellular stores or enters beta cells via routes other than VDCC. We addressed the question by using acetylcholine (ACh) to mobilize intracellular Ca^{2+} and promote capacitative Ca^{2+} influx via voltage-independent, store-operated channels that are activated by depletion of intracellular calcium stores.

Materials and methods: After 1–2 days of culture, normal mouse islets were treated with 1 μM /l latrunculin B to depolymerize or 1 μM /l jasplakinolide to polymerize beta cell microfilaments. They were then perfused to measure insulin secretion and $[\text{Ca}^{2+}]_i$ (fura-2 method). The proportion of globular/filamentous actin was measured biochemically.

Results: Filamentous actin (25% in control islets) was decreased to $\sim 5\%$ after latrunculin and increased to $\sim 95\%$ after jasplakinolide. In most experiments, islets were perfused with 10 mM/l glucose and 250 μM /l diazoxide (to hold beta cells hyperpolarized). ACh (100 μM /l) induced sharp and large peaks of $[\text{Ca}^{2+}]_i$ and insulin, followed by lower but sustained and reversible plateaus. Latrunculin did not affect $[\text{Ca}^{2+}]_i$ changes but doubled both phases of secretion. Jasplakinolide had little effect on first peak but suppressed second phase of ACh-induced secretion without attenuating the $[\text{Ca}^{2+}]_i$ signal. In Ca^{2+} -free medium, ACh induced rapid increases in $[\text{Ca}^{2+}]_i$ (mobilization from intracellular stores) and insulin secretion, but no sustained second phases. The $[\text{Ca}^{2+}]_i$ peak was slightly reduced by latrunculin and jasplakinolide, but only jasplakinolide decreased the insulin peak. After 30 min in absence of Ca^{2+} , reintroduction of CaCl_2 , in the presence of methoxyverapamil (a blocker of VDCC), was followed by an increase in islet $[\text{Ca}^{2+}]_i$ that was larger after pretreatment with ACh (indicative of capacitative Ca^{2+} entry) and unaffected by latrunculin or jasplakinolide. Insulin secretion also occurred, and was augmented 2x by latrunculin and diminished 2x by jasplakinolide as compared with controls. In other experiments Ca^{2+} entry through VDCC was induced by depolarization with KCl or tolbutamide. The ensuing increase in $[\text{Ca}^{2+}]_i$ was unaffected by latrunculin B or jasplakinolide treatment, but the resulting secretion of insulin was augmented by both agents.

Conclusion: The influence of beta cell actin microfilaments on insulin secretion differs with the source of triggering Ca^{2+} . Both disruption and stabilization increase the secretory response to Ca^{2+} entering via VDCC, whereas the response to intracellular Ca^{2+} mobilization is augmented only by microfilament disruption. Insulin secretion induced by capacitative Ca^{2+} entry is augmented by microfilament disruption and inhibited by microfilament stabilization. The results therefore suggest that distinct pools of insulin granules are implicated in response to Ca^{2+} from different sources.

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OP 47 Socio-economic aspects

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Implementation of integrated diabetes care in a diabetes care system for type 2 diabetes patients in primary care: an evaluation of resource use and costs

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Background and aims: Diabetes is associated with a high impact on health care use and costs. A strategy to improve the quality of care, manage the increasing demand for care and control the costs of care is the implementation of integrated diabetes care. Studies evaluating the effects and costs of integrated diabetes care have shown inconsistent results. In most studies a control group or information on costs on the societal perspective is missing. We evaluated care and costs on the societal perspective of integrated diabetes care compared with usual diabetes care for type 2 diabetes patients.

Materials and methods: Patients treated by the Diabetes Care System (DCS) receive integrated diabetes care. The DCS coordinates the care between involved health care professionals. Patients have a central role in the care and were stimulated to make their own choices regarding treatment and lifestyle behaviour. Diabetes nurses visit participating general practitioners to give feedback on patient and population level. A random sample of 253 diabetes patients, who were treated by the DCS were compared with 414 diabetes patients who received usual care according to current diabetes guidelines, generally organized in a single general practice. Patients reported received screenings and assessments and which health care professionals they had visited during the last year. Regression analysis was performed, estimating differences in costs between the DCS and usual care. Because of the skewed distribution of costs, we used bootstrapping methods (5000 replications) to estimate 95% confidence intervals (CI's) around the differences in costs. To take into account the (nonlinear) effect of diabetes duration on costs, we stratified for diabetes duration (1–2, 3–5 and ≥ 6 years).

Results: A higher proportion of DCS patients received medical examinations according to current diabetes guidelines (e.g. foot screening: 98.4% vs. 81.5%, $p < 0.001$). Compared to usual care patients treated by the DCS had fewer consultations with specialists in secondary care. Direct costs were lower in the DCS compared to usual care, in patients with diabetes duration of 3 to 5 years (Euro: -261 (95% Confidence Interval (CI): -617 to 96)), and with diabetes duration of more than 5 years (Euro: -671 (95% CI: -1187 to -154)), but equal in patients with duration < 3 years. (Euro: 7 (95% CI: -125 to 121)) (Table).

Conclusion: The Diabetes Care System is associated with better diabetes care as well as lower health care costs.

Mean (SD) costs during three months according to diabetes care group		
	Integrated care	Usual care
Diabetes duration 1–2 years	n=60	n=67
Direct costs	250 (323)	249 (303)
Total costs	926 (3092)	372 (584)
Diabetes duration 3–5 years	n=65	n=113
Direct costs	257 (249)	538 (2072)
Total costs	510 (856)	888 (2561)
Diabetes duration > 5 years	n=128	n=234
Direct costs	363 (814)*	1037 (3867)
Total costs	529 (1087)*	1351 (4275)

Direct costs: costs of visits to health care professionals, laboratory tests, use of home care, hospitalizations and costs of integrated care

Total costs: direct costs and costs related to productivity loss

* Indicates a significant difference (< 0.05) between integrated care and usual care after adjustment for age, sex, educational level and work status.

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Impact of the secondary diagnosis of diabetes in inpatient outcomes: areas of strategic intervention

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Background and aims: Hyperglycemia in hospitalized patients is an indicator of poor outcome, increased length of stay (LOS) and cost. On this regard, more knowledge is necessary in order to identify strategic areas of intervention.

Materials and methods: 62,055 admissions of a tertiary care center were studied with discharge summaries analysis software (Clínical*). Patients with the secondary diagnosis of diabetes (SDD) identified by Diagnostic Related Groups (DRG) in the Minimum Basic Set of Data (MBSDD) were compared with those without it. Variables considered were age, LOS, complexity (DRG weight), number of diagnostics and procedures per admission, major diagnostic category (MDC), unit of admission, rate of complications, readmission rate, patient safety indicators (PSI), and mortality. Obstetrics, Pediatrics and Diabetes departments were excluded.

Results: Diabetes was identified in 21% of episodes (12,751). Higher age (71 vs 59 years) and LOS (8.7 vs 7.3 days) were observed in patients with SDD. More difference in LOS was detected in the range between 26 and 65 years old. No differences in LOS were shown in older than 65yo. SDD was related to a greater DRG weight (1.95 vs 1.81), number of diagnostics (8.29 vs 5.78) and procedures (5.18 vs 4.77). 7 MDC accounted for 84% of the diabetic cases (44% were gathered in circulatory and respiratory system, 40% were distributed into gastrointestinal, kidney-urinary tract, hepatobiliary-pancreatic system, nervous system and musculoskeletal system). Only differences in surgical complications were observed (3.34% vs 2.83%). Readmissions were higher among diabetics (11.67% vs 8.24%). Regarding PSI; pressure ulcers (5.97_{0/00} vs 3.56_{0/00}), death in low mortality DRG (Dlm-DRG) (9.46_{0/00} vs 2.13_{0/00}), post-operative bleeding and hematomas (2.39_{0/00} vs 1.09_{0/00}) and postoperative pulmonary embolism or deep vein thrombosis (DVT) (4.43_{0/00} vs 2.69_{0/00}) were more frequent in diabetics. No differences in global mortality were observed. Major differences in LOS were observed in the following GRDs: hip joint replacement, bronchitis and asthma, urethral and transurethral procedures, other respiratory diagnoses with complications, acute myocardial infarction, hip and femur procedures excluding joint replacement, pulmonary edema and respiratory failure, and kidney-urinary tract infections. Surgical complications were more prevalent in Hematology, Oncology, Otolaryngology, Orthopedics, Urology and Ophthalmology units.

Conclusion: Diabetic patients have higher LOS, complexity of acute hospitalization episodes, number of procedures, surgical complications and readmissions. Furthermore, diabetes is associated to a higher percentage of PSI. According to our results, areas of strategic intervention would be Cardiology, Orthopedics, Urology and Respiratory units. Furthermore, surgical diabetic patients with hematologic and oncologic conditions should also deserve special attention. Also, a special consideration to Dlm-DRGs is warranted. Finally, pressure ulcers, DTV and bleeding prevention programs should consider diabetes as a risk factor.

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Duration of time off paid employment for conditions commonly associated with diabetes-related complications

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Background and aims: Diabetes mellitus (DM) is associated with a high and growing burden on health care systems and society. Days of paid work missed due to complications are part of the economic burden of DM. A study was undertaken to elicit time off work associated with DM-related complications in the working population in Sweden.

Materials and methods: The study utilised the STORE database which is maintained by the Swedish Social Insurance Agency and records all claims for sick pay in Sweden. Sick pay may be claimed for sick periods lasting 14 days or longer including prevention, extended and continued sickness ben-

efit, rehabilitation and sickness due to work injury. We extracted episodes of sickness completed over a 12-month period between 1 October 2009 and 30 September 2010 for 16 conditions which are commonly associated with DM-related complications. Conditions were identified by 3 digit ICD-10 code. We extracted sick time (calendar days between the start and end of sick period) for all cases meeting our criteria and calculated mean, median and standard deviation of the number of sick days per episode for each condition.

Results: The most common conditions were related to coronary heart disease (AMI, angina heart failure) and to cerebrovascular accident (stroke events and post-stroke).

Estimated sick leave duration per DM-related condition

Condition (ICD-10)	Number of episodes (*, <10)	Duration of sick leave in calendar days		
		Mean	SD	Median
Post stroke	454	618	477	556
Renal dialysis	14	564	528	514
Renal transplant	330	461	510	252
Stroke event	2,514	403	419	233
Peripheral vascular disease	110	364	434	160
Heart failure	670	296	358	149
Severe vision loss	68	281	337	124
Cataract	*	266	349	266
Neuropathy / Amputation	14	231	441	59
Foot ulcer	136	183	271	67
Gangrene	*	182	241	75
Angina	1,120	159	290	62
Myocardial infarction	2,463	93	189	45
Metabolic acidosis event	22	75	136	28
Major hypoglycemia event	*	37	18	40

Conclusion: Data from Swedish Social Insurance Agency show that work loss due to major events associated with DM is frequent and extended. These estimates may underestimate burden in people with DM as the database does not collect absences of less than 14 days, also data are not limited to people with DM, and need to be adjusted for workforce participation. These new data will permit healthcare payers and decision makers to estimate with greater precision the impact of diabetes interventions on productivity loss.

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Comparative US claims data in type 2 diabetes mellitus patients who have undergone bariatric procedures

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Background and aims: Earlier this year, the use of bariatric surgery as a weight loss and treatment option for obese patients with type 2 diabetes (T2D) was the subject of a position paper by the International Diabetes Federation. To help inform patients and providers of potential costs in patients with T2D, we conducted a longitudinal study in the United States to measure the intermediate-term health care consumption and costs associated with a restrictive out-patient procedure, laparoscopic adjustable gastric banding (LAGB), and malabsorptive surgery, Roux-en-Y bypass (RYGB). For these patients, medical and prescription claims may serve as proxy measures to disease state severity.

Materials and methods: Utilizing Source® Lx integrated claims repository data from 2002-09, physician practice, pharmacy, and hospital claims identified RYGB and LAGB patients by CPT or ICD-9 code. Patients were screened for T2D using ICD-9 code and evaluated up to 4 years(yr) before and after their surgical procedure. Care consumption was analyzed across prescription claims, physician office visits, and hospital visits. Costs of care were measured through out-of-pocket prescription expenses and total cost, submitted physician office and hospital charges, and total healthcare charges.

Results: All subjects experienced a similar increase in metrics of care consumption from 4 years pre-procedure up to date of the procedure. However,

in the years following surgery, differences were observed in health care consumption and costs between RYGB and LAGB:

Avg. Per Patient Utilization or Cost	1 yr pre-op (baseline (BL))		1 yr post-op		4 yr post-op			
	RYGB	LAGB	RYGB	LAGB	RYGB		LAGB	
					Value	Δ from BL	Value	Δ from BL
# T2D	2.23	2.14	1.67	1.53	1.64	-26%	1.58	-26%
Medications						p=.0001		P=.017
T2D Rx	10.7	9.5	5.2	4.3	6.5	-39%	4.0	-58%
Claims						p=.0001		p=.0001
Rx Claims	28	21	19	16	20	-30%	13	-46%
(total cost USD)	(2200)	(2280)	(1530)	(1730)	(1540)	p=.0001	(1230)	p=.0038
Office Visits	10	8.5	8	8	6	-4%	4	-54%
(charges USD)	(2590)	(2110)	(1790)	(1770)	(2480)	p=.72	(970)	p=.0001
Hospital Visits	5.1	4.8	6.3	5.1	5.8	+81%	3.6	+3%
(total charges USD)	(7963)	(8165)	(9934)	(8862)	(14435)	p=.0001	(8448)	p=.91
Total Healthcare charges (USD)	7743	7630	7245	4833	9254	+20%	4288	-44%
						p=.31		p=.001

Conclusion: These data demonstrate that both surgical procedures are similarly effective at reducing both care consumption and cost in T2D patients in the short-term. Of note, longer-term costs then increased in patients undergoing RYGB, driven by increased hospital cost. In contrast, patients with LAGB had a continued reduction in health care cost and utilization. It is hypothesized that the increased 4 year costs of RYGB are related to surgical complications and to morbidity caused by malabsorption of various micro-nutrients. These differences suggest the need for additional study.

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OP 48 Devices

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Efficacy and safety of continuous glucose monitoring systems vs self-monitoring blood glucose in patients with type 1 diabetes mellitus: a systematic review and meta-analysis

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Background and aims: There is an ongoing debate concerning the role of continuous glucose monitoring (CGM) systems in diabetes care. Several studies regarding the efficacy and safety of CGM have recently been published, so there is a great need to summarize their results in a comprehensive way. We performed a systematic review and meta-analysis to evaluate the effect of CGM on glycaemic outcomes as compared with self-monitoring of blood glucose (SMBG) with conventional glucometer readings in type 1 diabetes mellitus (T1DM).

Materials and methods: The assessment was based on randomized controlled trials (RCTs) identified by means of a systematic literature search in medical databases (Medline, EMBASE, Cochrane Library and others) up to January 2010. Studies met the inclusion criteria if they compared CGM vs. SMBG in T1DM patients on an intensive insulin regimen (continuous subcutaneous insulin infusion or multiple daily injections). It was required that the same regimen be used in both arms. Weighted mean difference (WMD), standardized mean difference (SMD) or odds ratio (OR) were calculated with a 95% confidence interval. Subgroup analysis was also performed regarding patient age (children, adolescent, adults), glycaemic control (measured by mean HbA_{1c} at baseline), type of CGM system.

Results: We identified 13 trials including together 1125 patients followed for at least 3 months. A meta-analysis of 9 RCTs demonstrated lower glycated hemoglobin (HbA_{1c}) level at the end of follow up in the CGM group compared with the SMBG one (WMD = -0.31 [-0.50; -0.12]). Pooled data of 13 RCTs showed greater HbA_{1c} change from baseline in favor of CGM (WMD = -0.26 [-0.34; -0.18]) and these results were consistent across most analyzed subgroups. Moreover, in the CGM group, a higher percentage of patients achieved predefined target HbA_{1c} (29% vs. 16%; OR = 2.13 [1.39; 3.26]) as well as HbA_{1c} reduction by at least 10% (22% vs. 9%; OR = 2.95 [1.53; 5.71]). It was also shown that incidence of any hypoglycaemic episodes was lower in the CGM group than in the SMBG one (SMD = -0.33 [-0.56; -0.10]). No differences between CGM and SMBG were revealed with respect to the percentage of patients with severe hypoglycemia or duration of hyperglycemic episodes (>180 mg/dL) or hypoglycaemic incidents. The safety analysis showed that CGM was well tolerated. Reported adverse events (AEs) included adverse reactions at the sensor implantation site (tenderness, redness). No severe AEs were observed.

Conclusion: The use of CGM may contribute to the improvement of glycaemic control and reduction of incidence of hypoglycaemic episodes in T1DM. Supported by: Medtronic Poland

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Efficacy of a bi-hormonal closed loop system to control postprandial and post exercise glucose excursions

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Background and aim: Continuous Subcutaneous Insulin Infusion (CSII) and Continuous Glucose Monitoring (CGM) sensors combined with an insulin and optionally a glucagon delivery algorithm constitute a closed loop system or artificial pancreas. The aim of this pilot study was to test the efficacy of a bihormonal closed loop system compared to CSII, challenging the system with two meals and an exercise bout. To our knowledge, this is the first report on exercise as a challenge for a closed loop system.

Materials and methods: Ten subjects with type 1 diabetes treated with CSII underwent a standardized day on three different occasions: a 40 grams carbohydrate breakfast was followed two hours later by 30 minutes of moderate exercise at 75–80% intensity of the heart rate reserve and one and a half hours later by a standardized 60 grams carbohydrate lunch. The first day served as control with open loop control, the second day as learning experiment for the algorithm to set individual patient parameters and the third day to compare the closed loop to the control day. Venous glucose was measured as refer-

ence every 30 minutes until 4 hours after lunch. In case of hypoglycaemia < 3.5 mmol/l the system recommended food intake. The closed-loop system consisted of two Medtronic CGM's (one primary and one back-up in case of failure of the first sensor) and two D-Tron+ pumps (Disetronic Medical Systems) for subcutaneous insulin and glucagon administration, all connected to a personal computer.

Results: Eight subjects were included, 6 were male. Mean (SD) age was 55.8 (10.0) years, mean HbA_{1c} 7.7 (0.9) %, mean duration of diabetes 33.8 (11.2) years and mean duration of pump use 9.5 (3.5) years. No significant differences in mean (minimum - maximum) venous glucose concentrations were seen; 8.5 mmol/l (5.4 - 11.7) in open loop compared to 8.2 mmol/l (6.3 - 9.4) in closed loop, $p = 0.76$. The median (minimum - maximum) AUC of the sensor glucose concentration in open loop was 3,973.8 mmol/l*minutes (2,669 - 5,253.2) compared to 4,300.3 mmol/l*minutes (356.2-4,867.8) in closed loop, $p = 0.78$. No differences in mean (minimum and maximum) insulin dosages were seen: open loop 23 (10.9 - 45) IU insulin, closed loop 30.6 (12 - 60.3) IU insulin, $p = 0.08$. On the control day the system advised food intake on two occasions, compared to four times on the intervention day, three of which in the post-exercise period.

Conclusion: Closed-loop glucose control was comparable to open loop glucose control with CSII. Hypoglycaemia was infrequent, but it was numerically more often in closed loop, despite glucagon use. Exercise is an important challenge for closed loop control.

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Microcirculation and its relation with continuous subcutaneous glucose sensor accuracy in cardiac surgery patients in the intensive care unit

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Background and aims: Glucose regulation is important in critically ill patients following cardiac surgery. Continuous subcutaneous glucose monitoring (CGM) could help achieving glucose targets and preventing hypoglycemia. However, the accuracy of the systems is uncertain in these patients. We hypothesized that CGM accuracy is influenced by microcirculatory variables. Therefore, we investigated the microcirculation and its relation with accuracy of two CGM devices in patients after cardiac surgery.

Materials and methods: We performed a prospective, observational study in a 20-bed intensive care unit (ICU). We included 60 patients who were about to undergo cardiac surgery. Two CGM devices (Guardian Real-Time, Medtronic Minimed; FreeStyle Navigator, Abbott Diabetes Care) were placed subcutaneously in the abdominal wall before surgery. Relative absolute deviation (RAD) between sensor and arterial reference glucose was calculated to assess CGM accuracy every two hours. Microcirculation was measured by microvascular flow index (MFI), perfused vessel density (PVD) and proportion of perfused vessels (PPV) using sublingual sidestream dark-field (SDF) imaging, and tissue oxygenation (StO₂) obtained with near-infrared spectroscopy. Associations were assessed by a linear mixed-effects model for repeated measures.

Results: Thirty-two patients underwent only a CABG procedure. Median (IQR) APACHE IV PM was 0.01 (0.003-0.02). Median (IQR) RAD was 11% (8-16) for the Navigator and 14% (11-18) for the Guardian ($p = 0.05$). StO₂ significantly increased during ICU admission (maximum 91.2% [3.9] after 6 hours) and decreased thereafter, stabilizing after 20 hours. The increase in StO₂ was accompanied by a decrease in PVD. MFI and PPV did not show a time effect (median [IQR] MFI 2.8 [2.7-2.9], PPV 0.97 [0.96-0.99]). Microcirculatory parameters were not associated with sensor accuracy. For the Navigator lower peripheral temperature ($b = -0.008$, $SE = 0.003$, $p = 0.003$), higher APACHE IV PM ($b = 0.017$, $SE = 0.004$, $p < 0.001$) and higher age ($b = 0.002$, $SE = 0.001$, $p = 0.037$) and for the Guardian lower peripheral temperature ($b = -0.006$, $SE = 0.003$, $p = 0.048$) were significantly associated with decreased sensor accuracy.

Conclusion: This study showed that microcirculation was impaired in patients after cardiac surgery during the first hours of ICU admission, determined by a decrease in PVD and an increase in StO₂, but to a limited extent only compared with septic patients and healthy controls. None of the microcirculatory parameters showed a relationship with continuous glucose sensor accuracy. Parameters that were associated with worse sensor accuracy were lower peripheral temperature (both sensors) and higher APACHE IV PM or higher age (Navigator). These results support CGM use in cardiac surgery patients characterized by low severity of illness. Further studies need to as-

sess the influence of microcirculatory changes on sensor accuracy in more severely ill, e.g. septic, patients.

Clinical Trial Registration Number: NTR1790

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A wireless, fully implantable continuous glucose sensor

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Background and aims: This sensor system implements a proprietary fluorescent transducer, optical sensor readout, and a wireless telemetry interface to enable a continuous glucose monitoring platform. The sensor part of this system is implanted into the subcutaneous space in the wrist and remotely powered through a wristwatch-based reader system. The glucose sensitive fluorescent indicator is excited with the embedded LED and then transduced through the filtered on-board photodiodes, which segments the various wavelengths of the fluorescence into signal and reference channel spectra. The sensor merges the optical signals with temperature transduction upon query from reader system, which externally provides power to the sensor during interrogation. A fully customized ASIC has been developed to merge this functionality into a multichip module that is the size of a swollen grain of rice and uses an on-site antenna that is loosely coupled to the reader of the external reader system. Overall, this sensor platform aims to provide high resolution, analytical glucose data that is transmitted wirelessly through the skin.

Materials and methods: The in-vitro testing of this sensor platform characterized the transducers baseline fluorescence, temperature sensitivity, dissociation constant, and transient response time to step changes in glucose. Part of the clinical investigations done with this platform includes long term implantation of the sensor system in primates with bi-weekly glucose clamp sessions and YSI blood samples drawn every 5 minutes.

Results: The overall function of the sensor system has proven to show continuous and automated tracking of ISF glucose through the wireless, passive telemetry interface. Watch-to-sensor communication and the implemented optical interface electronics designed into the ASIC have demonstrated a stable platform for wireless interrogation through the dermis. The transduction performance of this sensor platform shows an in-vitro sensor response time (t_{90}) of less than five minutes with a glucose dissociation constant of ~ 400 mg/dL, which provides an optimized accuracy range for detecting hypoglycaemia and hyperglycaemia events. The data acquired in monkey testing shows a high level of glucose accuracy, with a MARD of less than 7% for more than 2500 data points. A Clark Error Grid analysis of glucose excursions ranging between 40–340 mg/dL shows 94.4% and 5.6% of the data in the A and B regions, respectively. The pre-clinical sensor performance also supports the goals for this system in achieving implant times greater than six months.

Conclusion: This platform has been developed to provide transcutaneous communication and power delivery to an implanted fluorescent glucose sensor. The implanted performance has shown a high degree of glucose accuracy throughout primate pre-clinical studies and is currently moving into initial human clinical trials.

PS 001 Prediction of type 2 diabetes

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Factors associated with early versus late conversion to diabetes in community dwelling Danes

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Background and aims: The Danish National Diabetes Register (DNDR) enables sensitive and specific identification of new-onset diabetes by combining national registers. The aim was to evaluate factors associated with late versus early conversion to diabetes in community dwelling subjects.

Materials and methods: It has been shown that DNDR detects three times as many new-onset diabetes cases as do the ICD (International Classification of Diseases) system during a follow-up of median 13.8 years in the MONICA10 cohort (MONitoring trends and determinants of Cardiovascular disease) of 41 to 71 years old non-diabetic Danes. Out of a total of 2493 non-diabetic subjects in the MONICA10 cohort 186 were identified as new-onset diabetes during the follow-up period. Arbitrarily, early converters to diabetes were defined as those subjects converting within 8 years from baseline measurements.

Results: Early ($n=79$) versus late ($n=107$) converters displayed similar distribution of age, sex, body mass, waist circumference, physical activity, alcohol consumption, markers of low-grade inflammation (CRP and soluble UPAR), triglyceride, fasting insulin and markers of early organ damage as carotid plaques, aortic pulse wave velocity and urine albumin creatinine ratio. But early conversion to diabetes associated with a small increase in fasting plasma glucose (5.5 mmol/L vs. 5.2, $P<0.05$) and a diagnosis of hypertension (68% vs. 51, $P<0.05$) and inversely to smoking habits (current smoking, 34% vs. 50, $P<0.05$).

Conclusion: Minor increase in plasma glucose and hypertension but not measures of dyslipidemia, current smoking, early cardiovascular organ damage, low-grade inflammation or physical inactivity differentiated those prediabetic subjects who converted early versus late to diabetes. Intervention studies should evaluate the effect of addressing fasting glucose and blood pressure to postpone or prevent development of diabetes.

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The Leicester Practice Computer Risk Score - an automated tool for identifying those with impaired glucose regulation or type 2 diabetes mellitus using the new diagnostic criteria

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Background and aims: Modelling studies have suggested that early detection of impaired glucose regulation (IGR) and appropriate intervention can decrease morbidity and early mortality from cardiovascular disease. Risk scores utilising routinely collected data can identify those at high risk for further diagnostic testing. To date no automated risk scores have been developed and validated for using the new World Health Organization (WHO) 2011 diagnostic criteria for type 2 diabetes mellitus (T2DM).

Materials and methods: We used data on 6,390 subjects aged 40–75 from the ADDITION-Leicester (UK) screening study from a multi ethnic setting. All participants were given a 75g Oral Glucose Tolerance Test (OGTT). We developed logistic regression models for predicting IGR (impaired fasting glucose/impaired glucose tolerance/T2DM diagnosed according to the OGTT or HbA1c ≥ 6.5) from covariates which are routinely stored electronically in primary care. Using the best fitting model we developed the Leicester Practice Computer Risk Score by summing the beta coefficients. We externally validated the tool using data from 3,225 subjects aged 40–75 screened as part of a

second screening study. For both datasets the sensitivity, specificity, and positive and negative predictive values were calculated for cut points on the score for identifying the top deciles of risk. The discrimination was assessed using the area under the receiver operating characteristic (ROC) curve. Calibration was assessed using the Hosmer and Lemeshow (HL) statistic.

Results: The final model has an area under the ROC curve of 70.2% (95% CI 68.5–71.9%) and includes age, ethnicity (White European versus other - predominantly South Asian), sex, family history of T2DM, prescribed antihypertensive therapy and body mass index. There were no statistically significant two-way interactions. Polynomial terms were considered for age and BMI but this did not improve the fit of the model. This model gave good agreement between the observed and predicted estimates (HL 3.62, $p=0.89$). In the test data set using a cut-point which identifies the top 25% at risk identified 421 out of 774 who had IGR/T2DM and gave a sensitivity of 54.4% (95% CI 50.8–57.9%), specificity 72.8% (95% CI 71.0–74.5%), positive predictive value 38.7% (95% CI 35.8–41.7%), and negative predictive value 83.5% (95% CI 81.8–85.0%). This cut point can also be used to rule out T2DM alone, negative predictive value 92.4% (95% CI 91.2–93.5%), sensitivity 55.9% (95% CI 50.6–61.0%), specificity 69.1% (95% CI 67.4–70.8%), and positive predictive value 18.8% (95% CI 16.6–21.3%).

Conclusion: The Leicester Practice Computer Risk Score can be used to reliably identify those at high risk of IGR in multi ethnic populations. This is the first automated risk score based on the 2011 updated WHO diagnostic criteria for T2DM.

Leicester Practice Computer Risk Score =

0.040557 * age in years
+ 0.183559 if male, 0 otherwise
+ 0.081417 * BMI (kg/m²)
+ 0.753563 if South Asian, 0 otherwise
+ 0.536155 if prescribed antihypertensives, 0 otherwise
+ 0.468519 if family history of diabetes, 0 otherwise

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Protein peak information improves the prediction of type 2 diabetes: the Whitehall II study

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Background and aims: Improving prediction of type 2 diabetes (T2DM) is essential for refining diabetes prevention strategies. A number of protein biomarkers have recently been suggested to predict T2DM and advances in mass spectrometric methods now allow for the quantitative study across the entire human proteome. We examined whether information on protein peak signals improved prediction of incident T2DM beyond established lifestyle and biochemical risk factors for T2DM.

Materials and methods: The analysis is based on serum samples from 329 of the 560 participants in a nested case-control study within the Whitehall II cohort. Participants were free of diabetes at baseline (1991–1994). The 111 cases developed T2DM during the 16 year follow-up period. The 218 controls were frequency-matched for age, sex, and BMI. Diabetes was defined by the OGTT. Data on 364 protein peaks were acquired by linear matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) in the 1–20 kDa range. The 70 peaks present in at least 5% of the participants were analyzed together with 33 known risk factors for T2DM including age, sex, obesity measures, employment grade, smoking status, physical activity, alcohol intake, family history of diabetes, use of anti-hypertensive or lipid-lowering medication, blood pressure, lipid profile, apolipoproteins and inflammatory markers. Two prediction models for incident T2DM were derived. Model 1: the 33 known risk factors for T2DM were ranked using random forest analysis and the 10 most informative factors were selected for further analysis. A classification tree for incident T2DM was derived based on these 10 factors. Model 2: the 70 peaks were ranked according to their

added discriminatory value to the first model using random forest analysis. A second classification tree was derived based on the first model and the 10 most informative peaks from the random forest analysis. Model performance with and without peaks was evaluated and compared using receiver operating characteristics (ROC) analysis and integrated discrimination improvement (IDI).

Results: The first model included systolic blood pressure, triglycerides and family history of diabetes. The model with protein peak data additionally included two of the 10 protein peaks and this increased the area under the ROC curve (AUC) by 8% ($p<0.01$) and IDI by 63%.

Conclusion: Information on protein peak signals improves prediction of incident T2DM beyond established lifestyle and biochemical risk factors.

AUC with 95% confidence interval (CI) and IDI

	AUC (95%-CI)	P-value for increase in AUC	Relative IDI
Model 1	0.67 (0.61;0.73)	reference	reference
Model 2	0.73 (0.67;0.78)	0.003	63%

IDI: integrated discrimination improvement

Model 1: systolic blood pressure, triglycerides and family history of diabetes

Model 2: model 1 and two protein peaks

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Long-term risk of type 2 diabetes and measures of overall and regional obesity: the European InterAct study

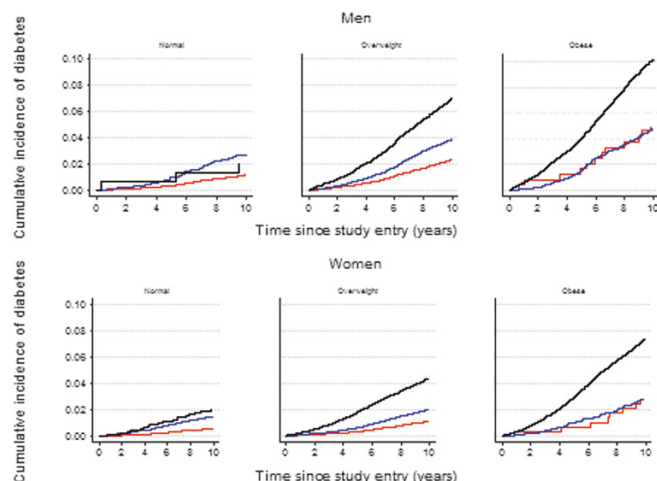
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Background and aims: Individual lifestyle interventions can reduce diabetes risk, but need to target those at highest risk. Current clinical practice relies on measurement of body-mass index (BMI), but waist circumference (WC) provides a simple and reliable measure of fat distribution that may add to diabetes risk prediction. Previous studies have not been powered to precisely estimate the absolute risk of diabetes to identify population subgroups in whom measurement of waist circumference would be most informative. We assess the clinical usefulness of measuring WC in normal weight, overweight and obese individuals.

Materials and methods: The InterAct case-cohort study was conducted in 26 centres in 8 European countries and consists of 12,403 incident T2D cases and a stratified subcohort of 16,154 individuals identified amongst 340,234 EPIC participants followed-up for 3.99 million person-years. Hazard ratios for T2D were estimated using Prentice-weighted Cox-regression and random effects meta-analyses. Cumulative incidences were estimated using Kaplan-Meier methods.

Results: BMI and WC were independently associated with T2D. The cumulative 10-year T2D incidence (figure 1) in normal weight men and women was $\leq 2.7\%$ and 2.0% , irrespective of their WC. In overweight individuals, WC distinguished those with incidence rates comparable to normal weight from those with rates equivalent to obese individuals. The cumulative 10-year incidence for men with normal, moderately increased, or large WC was 2.3% , 3.9% and 7.0% in overweight and 5.0% , 4.8% and 10.2% in obese men, respectively. Corresponding figures were 1.1% , 2.0% and 4.4% in overweight and 2.8% , 2.7% and 7.4% in obese women.

Conclusion: A substantial proportion of overweight individuals with a large WC will develop T2D over a 10-year period. Current recommendations should target this group, whose risk is comparable to obese individuals and who may benefit from early lifestyle intervention. Measurement of WC can guide clinical decision making in overweight individuals. Figure 1. Cumulative incidence of T2D over 10 years by sex, BMI and waist groups.



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Risk of diabetes and cardiovascular events in persons with early glucose metabolism impairments

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Background and aims: Russia ranks fourth with regards to number of patients with diabetes (by IDF data) and prevalence reaches 9%. Aim is to assess relative risk (RR) of type 2 diabetes (T2DM), overall and acute cardiovascular mortality and cardiovascular events in persons with impaired fasting glucose (IFG) and impaired glucose tolerance (IGT).

Materials and methods: According to population based study among 2508 adults, the 3-year risk of T2DM, overall and acute cardiovascular mortality and cardiovascular events (fatal and nonfatal myocardial infarction and stroke, coronary heart disease) was estimated in people with glucose metabolism abnormalities (GMA): IFG, IGT, IFG+IGT diagnosed in 2006 in comparison with normal glucose tolerance. RR and regression coefficient (B) was calculated using Cox-regression analysis. RR of T2DM, cardiovascular events was adjusted for age, sex, BMI, systolic blood pressure (SBP), smoking.

Results: Prevalence of GMA was 24.9%, including IFG (8.8%), IGT (5.1%), IFG+IGT (3.8%) and first time diagnosed T2DM (7.2%). In 3 years highest percent of transformation to T2DM and adjusted RR of T2DM were in people with IFG+IGT (33.3% and 11.2[3.93–31.65], $p < 0.01$, correspondingly). The lowest percent of transformation to T2D and RR of T2DM were in people with isolated IGT (10.3% and 3.92 [1.11–13.90], $p = 0.034$). Adjusted RR of cardiovascular mortality was significantly 3.2-fold higher among people with IFG. People with IGT and newly diagnosed T2D had significantly 3.6-fold and 2.3-fold greater risk of overall mortality. RR of cardiovascular events was significantly increased 2.2-fold among people with IFG and 2.7-fold in persons with newly diagnosed T2D, people with IFG+IGT also showed 2.6-fold increase of cardiovascular events risk, but it was not significant.

Conclusion: 3 year risk of developing T2DM is not equal in persons with different early GMA: it is highest among persons with IFG+IGT and lowest among persons with isolated IGT. IFG increased 3-year risk of acute cardiovascular mortality.

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Risk-stratified screening for type 2 diabetes in adult subjects: results from Hungary

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Background and aims: The prevalence rate of Type 2 diabetes mellitus has been increasing worldwide. The early diagnosis of diabetes is of great im-

portance in order to improve the late prognosis of the disease. Due to cost-effectiveness, screening should primarily be implemented in subjects at high risk to glucose intolerance.

Materials and methods: A risk-stratified nationwide screening procedure was performed by the Hungarian Diabetes Association in collaboration with general practitioners (GPs) in adult subjects without known diabetes. The screening was performed in a two steps manner. At first step, the Hungarian version of the validated FINDRISC questionnaire (maximum score 26) was filled out by subjects while waiting for GP. At second step, a standard oral glucose tolerance test (OGTT) with 75 g glucose was performed in subjects with a score of ≥ 12 . Blood glucose values were measured in venous blood samples. The classification of glucose intolerance was based on the report from the WHO, 2006.

Results: As a total, 56,684 subjects (60.5% women, 40.5% men) were screened between 01, Sept 2010 and 01, March 2011. Out of 22,581 subjects with a score of ≥ 12 , 2,403 subjects refused to perform OGTT. In the remaining 20,178 subjects 10,429 (51.7%) had normal glucose tolerance, while 9,749 (48.3%) had abnormal glucose tolerance as follows: impaired fasting glycaemia (IFG) 2,797 (13.9%), isolated impaired glucose tolerance (IGT) 1,731 (8.6%), IFG+IGT 1,851 (9.2%) and previously unknown diabetes mellitus 3,370 (16.7%) subjects. Glucose intolerance of any degree proved to be more prevalent in men than in women (53.2% versus 45.3%, $p < 0.001$). If the score value requiring OGTT was set at higher level (≥ 15 or ≥ 21), the proportion of subjects with any degree of glucose intolerance significantly increased (58.7% and 75.1%, respectively).

Conclusion: The risk-stratified screening procedure proved to be simple and effective for detecting early impairment of the carbohydrate metabolism, therefore, its nationwide implementation should be considered in Hungary. Clinical Trial Registration Number: 10835-0/2010-1018EKU (518/PI/010) Supported by: Ministry of Health

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Association between protein signals and type 2 diabetes incidence: the Whitehall-II study

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Background and aims: Predicting type 2 diabetes (T2DM) in early and progressive stages of disease development is essential for refining diabetes prevention strategies. Proteomic technologies allow for identification of novel proteins which reflect the pathophysiological changes in the lead up to diabetes. Our aim is to assess the association between protein signals and diabetes incidence.

Materials and methods: We analysed serum samples from 305 participants in a nested case-control selection within the Whitehall-II study. All participants were free of diabetes at baseline (1991–1994). Cases (N=100) developed T2DM during the follow-up period of 16.0 years; controls (N=205) were frequency-matched for age, sex, and BMI. Data on 36 4 peaks were acquired by linear matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) in the 1–20 kDa range. The 70 peaks present in at least 5% of the participants were ranked using random forest analysis. The 10 highest ranking peaks were selected for further analysis. Classification trees were used to assess the best binary split in peak intensity value for diabetes incidence. Odds ratios for diabetes in the group with peak intensities above the split were calculated using logistic regression for different levels of confounder adjustment.

Results: Table 1 presents the odds ratios for diabetes for the 10 highest ranking peaks. Most odds ratios were robust to adjustment for age, sex, BMI, smoking and systolic blood pressure.

Conclusion: We detected several peaks whose associated proteins may reflect mechanisms involved in diabetes pathogenesis. Further research is needed to determine the identity and functions of these proteins. Our study exemplifies the utility of an approach which combines proteomic and epidemiological data.

Table 1. Odds ratios for incident diabetes for different levels of confounder adjustment

	fraction (%) above intensity split value	Model 1	Model 2	Model 3
Peak 1	5	6.21 (1.93;20.04)	6.85 (2.07;22.71)	7.07 (2.08;24.03)
Peak 2	51	2.53 (1.54;4.16)	2.51 (1.53;4.14)	2.37 (1.42;3.96)
Peak 3	20	1.86 (1.05;3.31)	1.84 (1.03;3.28)	1.69 (0.93;3.08)
Peak 4	5	3.28 (1.13;9.49)	3.51 (1.20;10.3)	3.72 (1.23;11.25)
Peak 5	70	2.31 (1.30;4.11)	2.33 (1.31;4.15)	2.19 (1.21;3.97)
Peak 6	91	0.42 (0.19;0.92)	0.42 (0.19;0.95)	0.38 (0.16;0.86)
Peak 7	4	7.48 (2.01;27.84)	7.62 (2.03;28.52)	9.77 (2.51;37.97)
Peak 8	21	1.92 (1.09;3.36)	2.02 (1.14;3.57)	1.90 (1.05;3.44)
Peak 9	6	3.50 (1.31;9.32)	3.87 (1.41;10.60)	4.34 (1.52;12.42)
Peak 10	7	2.97 (1.21;7.31)	3.02 (1.22;7.48)	3.26 (1.28;8.32)

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Validation of the FINDRISC score for prediction of type 2 diabetes in the Spanish population: the VIVA (variability of insulin with visceral adiposity) cohort study

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Background and aims: To describe the utility of the FINDRISC questionnaire as a risk predictor for Type 2 Diabetes (T2D) in the Spanish population. **Materials and methods:** Population-based cohort study (1,766 individuals 34–64 years; 58% women), randomly selected in 1997 and free of diabetes at baseline (FPG < 7.0 mmol/L and 2-h PG < 11.1 mmol/L and no treatment with any anti-diabetic medicine). Mean follow-up 11.5 years. New cases (incident) of T2D were defined by WHO criteria: FPG > 7.0 mmol/L or 2-h PG > 11.1 mmol/L or initiation of any anti-diabetic treatment during the follow-up. Two FINDRISC scores (FR) versions: the 8-item FR and the 7-item FR (without information on family history of diabetes) were analyzed. The probability of T2D was estimated using logistic regression analysis. The discriminatory power of the FR scale was calculated by the AUC analysis, for different cutoffs. Calibration analyses for the 8-item FR and 7-item FR models were performed grouping the FR score in 5 groups to calculate the observed: predicted probability ratio.

Results: The follow-up (OGTT, and clinical information on anti-diabetic treatment) was completed in 1,401 subjects (80%). During the follow-up 161 (10.3%) new T2D cases were identified. Discriminatory power of the 7-item FR test by ROC analysis was: 0.662 (95%CI: 0.621, 0.703), and 0.693 (95%CI: 0.649, 0.736) for the 8-item FR using the WHO definition for T2D. If only the information on “new anti-diabetic treatment” (n=58) is used as the outcome variable, the corresponding ROC values are: 0.731 (95%CI: 0.660, 0.801). The table shows the 10-year observed: predicted probability ratio of T2D grouped in 5 Ntiles.

Conclusion: The usefulness of the FR, both 7-item and 8-item, to predict T2D is discrete and overestimates the risk T2D in our population. The application of FR in a logistic model to our data, without using the FR cutoffs, might improve the predictive probability. Adjusting the weights of the variables in our data probably would improve its predictive ability.

Ntiles of FR	PObs 8-item FR	PPred 8-item FR	PObs 7-item FR	PPred 7-item FR
1	0.0563	0.8100	0.0476	0.7069
2	0.0766	0.9873	0.0915	0.9667
3	0.1193	0.9987	0.1063	0.9961
4	0.1207	0.9999	0.1856	0.9995
5	0.2809	1.0000	0.2120	1.0000

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Influence of non-response in a population-based diabetes screening study evaluated by the Swedish Prescribed Drug Register

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Background and aims: Bias due to non-response may lead to over- or underestimation of disease risks. The aim was to evaluate potential selective non-participation in Stockholm Diabetes Prevention Program (SDPP), and whether this may have introduced bias and thereby influenced results in our previous studies.

Materials and methods: A population-based cohort in Stockholm comprising 12952 men and 19416 women aged 35–56 years, was screened for self-reported diabetes and family history of diabetes (FHD) by a postal questionnaire. Of these, two groups of similar size, one comprising all individuals with FHD and the other individuals without FHD, all without diagnosed diabetes, were selected to a baseline health examination with oral glucose tolerance test in 1992–94 for men (n=3128) and 1996–98 for women (n=4821). Follow-ups of 2383 men and 3329 women were performed in 2002–04 and 2004–06, respectively. In the present study, record linkage to the Swedish Prescribed Drug Register was used, and the absolute risk for diabetes was estimated as filled prescriptions of insulin or anti-diabetic drugs (in 2005–2008) for the total cohort of responders and non-responders at the SDPP screening step, and for participants and non-participants at baseline and follow-up steps. Odds ratios (OR) with 95% confidence intervals (CI) were calculated for the risk of drug-treated diabetes.

Results: At the screening step, absolute risks for diabetes did not differ in response and non-response groups, men 8.7/7.7% and women 4.0/3.8%. ORs in non-responders compared to responders were 0.9 (0.7–1.1) in men and 1.0 (0.8–1.2) in women. At baseline, absolute risks were 8.5/7.2% in men and 3.8/3.2% in women among participants and non-participants, respectively; ORs 1.0 (0.8–1.3) and 1.0 (0.7–1.3). At follow-up, the absolute risk for diabetes was lower among participants than non-participants, men 4.4/6.2% and women 1.6/2.6%, adjusted ORs 1.4 (0.9–2.3) and 1.5 (0.9–2.4) for non-participants. However, analyses did not exhibit overestimation of follow-up risks associated with FHD, smoking, physical activity, socioeconomic position and psychological distress. For overweight, the ORs for diabetes in participants and non-participants in men were 3.2 and 4.1 respectively, and for obesity 10.7 and 12.0, as opposed to women where ORs for overweight were 3.1 and 1.5, and for obesity 13.7 and 2.8. This indicated differential follow-up non-participation for BMI related to diabetes.

Conclusion: There was no selective non-response at either screening or baseline steps suggesting that the results from SDPP are generalizable. At follow-up, non-participants had an overall higher risk of diabetes compared to participants. However, analysis of risks associated with specific exposures revealed in general no overestimations. For BMI, the results illustrated different patterns among men and women, indicating that a higher share of overweight or obese men with diabetes seemed to avoid a follow-up health exam. This could lead to an underestimation of the risk estimate in men for high BMI, and also underlines the importance of targeting this risk group. Conversely, in women the impact of high BMI could be overestimated.

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Low water intake and risk for new-onset hyperglycaemia

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Background and aims: Recent data indicate an independent association between plasma copeptin, a surrogate for vasopressin, and risk of diabetes mellitus. Despite the known influence of water intake on vasopressin secretion, no

study has investigated a possible association between usual daily water intake and incidence of hyperglycemia. Our objective was to determine the relationship between water intake and the risk for hyperglycemia in a French cohort. **Materials and methods:** Participants were 3615 French men and women, aged 30–65 years, with normal baseline fasting glycemia (FG), recruited in the 9-year follow-up D.E.S.I.R study (Data from an Epidemiological Study on Insulin Resistance Syndrome). They were offered health examinations every three years, including a self-administered questionnaire with reports of mean daily intake of water, wine, beer-cider and sweet beverages. Main Outcome Measures were Odds Ratios (ORs) and 95% Confidence Intervals (95% CIs) for the incidence of hyperglycemia (impaired FG or diabetes, i.e., FG ≥ 6.1 mmol/l or treatment for diabetes) according to water intake classes.

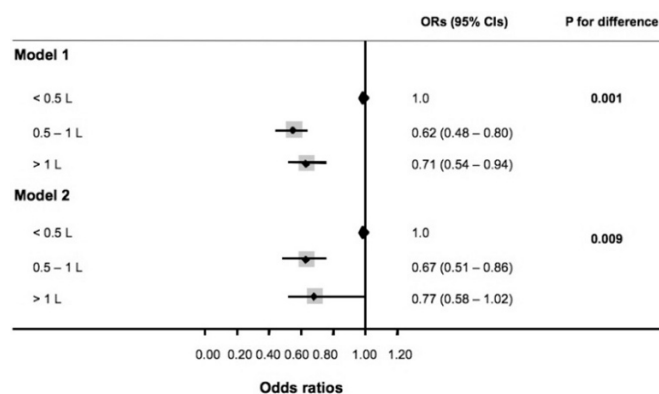
Results: During follow-up, there were 565 incident cases of hyperglycemia. After adjustment for confounding factors (sex, baseline age, body mass index, FG, physical activity, smoking status, triglycerides, HOMA-IR, total cholesterol, gamma-GT and familial history of diabetes) ORs (95% CI) for hyperglycemia associated with the volume of daily water intake (<0.5 L, 0.5 to <1.0 L, more than 1.0 L) were 1.00, 0.64 (0.49–0.83), and 0.73 (0.55–0.97), $P=0.003$ (Figure). The ORs were similar when stratified by various characteristics, including gender and alcohol consumption.

Conclusion: Self-reported water intake was inversely associated with the risk of developing hyperglycemia. Further studies are needed to establish whether vasopressin levels mediated this association and whether interventions to increase water intake may protect against hyperglycemia.

Figure: OR (95% CI) for the association between daily water intake at baseline and the risk of incident hyperglycemia.

Model 1: Adjusted for age, sex, BMI, baseline fasting glycemia, physical activity, smoking status, triglycerides, HOMA-IR, and total cholesterol.

Model 2: Further adjusted on self-reported mean daily volumes of beer-cider, sweet drinks, and wine consumed per day.



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PS 002 Genes, complications and pharmacogenetics

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Evaluation of the genetic effects of IGF2BP2 in the development of diabetic nephropathy

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Background and aims: Evidence has indicated that insulin-like growth factor 2 mRNA binding protein 2 (IGF2BP2) genetic polymorphism (rs4402960) is associated with type 2 diabetes (T2D) but not with type 1 diabetes (T1D). The IGF2BP2 gene is located on chromosome 3q27.2 and in a region linked to T1D, T2D and diabetic nephropathy (DN). In order to evaluate the genetic effects of IGF2BP2 in the development of DN, we have performed a genetic and biological study.

Materials and methods: Three cohorts, including T1D with and without DN (n=1139) in American Caucasians from the Genetics of Kidneys in Diabetes (GoKinD) study, T1D with and without DN (n=303) in Swedish Caucasians, non-diabetic control subjects, T1D, T2D with and without DN (n=1232) in Czech Caucasians were enrolled in the present study. Genotyping experiments for SNP rs4402960 in the IGF2BP2 gene was performed with TaqMan allele discrimination. IGF2BP2 mRNA expression levels in kidney tissues of db/db and control mice at the ages of 5, 14 and 26 weeks were determined by using real time RT-PCR.

Results: A strong association with T2D was found in the Czech population ($P=0.007$, OR=0.491 95% CI 0.290–0.831). There was also a borderline association with DN in Czech T2D patients ($P=0.040$, OR=0.571 95% CI 0.333–0.979). In the GoKinD population, a moderate association with DN was found in male T1D patients ($P=0.037$, OR=0.692 95% CI 0.490–0.978). No significant association with DN in Swedish T1D patients was detected due to limited sample size. IGF2BP2 mRNA expression in kidneys of db/db mice at the age of 5 weeks was increased compared to controls ($P=0.008$). The expression of IGF2BP2 in kidneys of control mice were dynamically increased from the ages of 5 weeks to 14 and 26 weeks. But, there was no significant difference among db/db mice at any age.

Conclusion: The present study provides the first evidence that IGF2BP2 expresses in kidneys of db/db mice and impaired in the progress of DN. IGF2BP2 has genetic impacts in the development of T2D. It may also be a susceptibility gene for DN in both T1D and T2D.

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KCNQ1 and genetic susceptibility to diabetic nephropathy in type 2 diabetes: replication in the Singaporean Chinese population

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Background and aims: KCNQ1 has recently been suggested to confer susceptibility to diabetic nephropathy in Japanese patients with type 2 diabetes. To replicate this finding, we analysed SNPs rs2237895, rs2237897 and rs2283228 within the KCNQ1 locus for association with diabetic nephropathy among type 2 diabetic Chinese patients residing in Singapore.

Materials and methods: A total of 940 Chinese type 2 diabetic patients were included in the study. Controls were normoalbuminuric while cases were divided into those with micro- and macroalbuminuria. Genotyping was performed using realtime PCR and invader assays as appropriate. Logistic regression was used in analyzing the associations between SNPs and diabetic nephropathy.

Results: SNPs rs2237897 and rs2283228 were significantly associated with macroalbuminuria but not microalbuminuria. Particularly, CC homozygotes for rs2283228 were more likely to have macroalbuminuria even after adjustment for significant patient covariates including glycaemic control, blood

pressure, BMI, triacylglycerols, and diabetes duration (adjusted odds ratio 4.27 [95% CI 2.11–8.66], $P < 0.001$). TT homozygotes for rs2237897 were also at a higher risk of macroalbuminuria although the statistical evidence was more moderate (adjusted odds ratio = 2.76 [95% CI = 1.22–6.25], $P < 0.02$).

Conclusion: Together with the previous Japanese study, our findings support the hypothesis that in addition to being an established type 2 diabetes gene, KCNQ1 may also confer genetic susceptibility to diabetic nephropathy as evidenced by macroalbuminuria among Asians.

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Allelic variations of superoxide dismutase 1 (SOD1) gene and diabetic nephropathy in type 2 diabetic patients

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Background and aims: Oxidative stress is involved in the pathophysiology of diabetic nephropathy (DN), and the enzyme superoxide dismutase 1 (SOD1) is essential for reactive oxygen species detoxification. Associations of SOD1 gene variants with DN have been reported in patients with type 1 diabetes. In this study, we assessed the impact of SOD1 allelic variation in the development and progression of DN in individuals with type 2 diabetes (T2DM) followed prospectively for renal events.

Material and methods: We studied unrelated French type 2 diabetic patients from the DIABHYCAR (n=3137) and the DIABHYCAR_GENE (n=607) cohorts. DIABHYCAR was a 6-year clinical trial conducted in men and women with T2DM selected on the basis of persistent microalbuminuria (urinary albumin excretion, UAE=20–200 mg/l) or macroalbuminuria (UAE>200 mg/l) without renal failure at baseline. The trial tested whether a low dose of ramipril able to reduce UAE would also reduce cardiovascular and/or renal events. A renal event was defined as the doubling of the serum creatinine levels or the requirement of haemodialysis or renal transplantation during follow-up. It occurred in 77 cases (2.46%). Results were negative regarding the drug effect and were published previously. The DIABHYCAR_GENE cohort was recruited concomitantly to DIABHYCAR and included men and women with T2DM presenting with normal UAE (UAE<20 mg/l). Seven SNPs (rs2173962, rs9974610, rs10432782, rs2070424, rs1041740, rs17880135 and rs202449), giving information on ~90% of the allelic variation of the haplotypic block containing SOD1 gene were analyzed. Genotype associations with DN were assessed by logistic regression analyses and by Cox proportional hazards survival regression analyses. Adjustments for clinical and biological parameters were carried out by including these parameters as covariables in the regressive model. The power to detect associations of the SNPs with DN at baseline and with incidence of renal events during follow-up was 0.98 and 0.74, respectively, for odds ratio or hazard ratio equal or higher than 1.5 and alpha=0.05.

Results: In a first step, participants were divided into 3 groups according to UAE at baseline: normal UAE (DIABHYCAR_GENE cohort), microalbuminuria and macroalbuminuria (DIABHYCAR cohort, both groups). Allele and genotype frequencies of the seven SNPs were similar in the 3 groups. Next, we assessed the impact of allelic variations on the renal outcomes of the original DIABHYCAR study. We have not observed any association of the SNPs with the incidence of renal events during follow-up neither in univariate analyses nor in complex models adjusted for sex, age, BMI, duration of diabetes, HbA1c levels, presence of arterial hypertension, history of myocardial infarction, treatment with ACE inhibitors or treatment group in the original DIABHYCAR study. In these analyses, only HbA1c, BMI, arterial hypertension and history of myocardial infarction at baseline were significantly and independently associated with the incidence of renal events at follow-up.

Conclusions: Allelic variations in the SOD1 gene were not associated with micro or macroalbuminuria nor with the deterioration of renal function in patients with T2DM followed prospectively for 6 years. These results suggest that SOD1 does not play a major role in the genetic determinants of DN in type 2 diabetes.

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Pilot application of multi-locus and time-to-event analysis to ascertain genetic risk factors for diabetic nephropathy and related adverse outcomes of diabetes mellitus

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Background and aims: Diabetic complications and namely nephropathy (DN) contribute significantly to morbidity and mortality of diabetic patients. Their development and progression is influenced by several factors incl. treatment/compensation of diabetes, presence of co-morbidities, life style and genetic factors. The latter one is being intensively studied as potentially very powerful tool of personalised medicine. Using follow-up data from prospective study of diabetic population of South Moravia region of Czech Republic the aim of our study was to analyse contribution of genetic factors to progression of DN (primary end-point) and to major non-fatal cardiovascular event (MCVE), cardiovascular (CVM) and all-cause mortality (ACM) as secondary end-points using combination of analytical approaches.

Materials and methods: Study comprised a total of 459 diabetic subject with variable stage of DN (i.e. normoalbuminuria, persist. microalbuminuria, proteinuria and ESRD) prospectively followed for a median of 39 [IQR 21 - 59] months. Following end-points were considered: [1] progression of DN by stage, [2] MCVE (non-fatal myocardial infarction or stroke, limb amputation), [3] CVM (fatal myocardial infarction, stroke, heart failure or sudden death) and [4] ACM. Presently, 86 single nucleotide polymorphisms (SNPs) in 37 candidate genes were genotyped. After quality control, 82 SNPs remained. For each endpoint and SNP, two association statistics were computed, chi-square in 2x2 tables of alleles and chi-square in 2x3 tables of genotypes. Associated P-values were assessed in 10,000 permutation samples with the Sumstat program. For nominal P values <0.05 a corresponding experiment-wise significance levels were corrected for multiple testing. Selected best associated variants were subsequently used in time-to-event analysis of individual end-points.

Results: Kaplan-Meier curves were constructed for four best associated SNPs for given end-points: (1) progression of DN between genotype groups of cytochrome b-245 242C/T (CYBA, rs4673) log rank test $P=0.03738$, (2) MCVE and ACM vs. coagulation factor V R506Q genotypes (F5, rs6025) log rank test $P=0.02539$ and $P=0.00151$, respectively, and (3) ACM vs. dimethylarginine dimethylaminohydrolase 2 449C/G (DDAH2, rs805305) and DDAH2 1151A/C (rs805304) genotypes, log-rank test marginally significant ($P=0.06030$ and $P=0.06710$, respectively). In case of CYBA 242C/T heterozygotes and TT homozygotes were risk genotypes for DN progression, in case of F5 R506Q heterozygotes were associated with MCVE and ACM.

Conclusion: In the pilot analysis of our on-going prospective study we identified F5 506RQ and CYBA 242CT+TT (marginally also DDAH2) genotypes as risk factors for progression of DN, MCVE and ACM. Using combined approach of multi-locus and time-to-event analysis it is possible to analyse large amount of genetic and clinical data generated in follow-up studies.

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Effect of vitamin D - therapy on insulin resistance and metabolic control in patients with type 2 diabetes mellitus and its pharmacogenetic analysis

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Background and aims: The effects of vitamin D (VD) on the metabolism have been mainly studied in non-diabetics. We investigated the influence of a six-month VD supplementation of 2000 U/d in patients with non-insulin-requiring type 2 diabetes mellitus (T2DM) and the pharmacogenetic impact of Vitamin D system polymorphisms (CYP27B1-rs10877012, VDR ApaI-rs7975323, VDR TaqI -rs731236 and CYP24A1-rs2248137).

Materials and methods: Patients with T2DM (n=86) were randomized in a double-blind, placebo-controlled study. During the first six months the pa-

tients received 20 drops Vigantol oil or placebo oil (medium chain triglycerides) once a week, followed by six months follow-up with measurements of 25D and 1,25D levels, PTH, calcium, phosphor, body weight, blood pressure, HbA1c and C-peptide. Vitamin D system gene polymorphisms were analysed by RFLP and RT-PCR. Wilcoxon-Mann-Whitney-U-Test was used for statistics.

Results: After 6 months of therapy the verum group ($n = 40$) 25D level had increased by a factor of 2.14 to a median of 28.4 ng/ml (71 nmol/l). The mean increase of 11.85 ng/ml ($p < 0.001$) in the verum group was significantly higher than in the placebo group. The PTH tended ($p = 0.08$) to decrease more in the verum group until the end of therapy. The verum group showed a non significant increase of calcium by 1.02 up to a median of 2.43 mmol/l but a significant increase of phosphor ($p = 0.04$) by 1.06 up to a median of 3.6 mg/dl. In the placebo group no change was seen, neither in calcium nor in phosphor. The changes in body weight and systolic blood pressure were not significant in any group. At baseline all patients with 25D levels > 20 ng/ml (52.5 nmol/l) ($n = 14$) had significantly lower HbA1c. The HbA1c was 0.35% ($p = 0.01$) lower than in patients with VD < 20 ng/ml ($n = 71$). After VD therapy all patients with 25D levels > 20 ng/ml showed higher C-peptide levels (by 0.95 ng/ml, $p = 0.01$). Pharmacogenetic differences were significant for the relative increase of 25D in carriers of CYP27B1 CC ($p < 0.001$) and AC ($p < 0.001$), VDR TaqI TT ($p < 0.001$) and Tt ($p < 0.001$) and VDR ApaI AA ($p = 0.005$) and Aa ($p < 0.001$) and CYP24A1 CC ($p = 0.005$), GG ($p = 0.004$) and CG ($p = 0.001$). Significant differences in relative increase of 1,25D were observed for CYP24A1 GG ($p = 0.05$). The difference in PTH suppression was significant for CYP27B1 AC ($p = 0.047$) and VDR ApaI Aa ($p = 0.042$) and for the increase of C-peptide with VDR ApaI AA ($p = 0.029$).

Conclusion: This study shows lower HbA1c at baseline as a function of the 25D status in patients with T2DM without insulin. Furthermore C-peptide increases significantly after six months of VD therapy. Some effects of this pilot vitamin D therapy appear to be under the influence of pharmacogenetic variation.

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Association study of six type 2 diabetes genes and glucose-lowering effect of sulfonylurea drugs

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Background and aims: Recently discovered gene polymorphisms associated with type 2 diabetes (T2D) unveiled the pathogenetic heterogeneity of T2D which may also influence the efficacy of antidiabetic treatment. Aim of our study was to determine the effect of gene polymorphisms associated with development of T2D on the parameters of glycemic control during treatment with sulfonylurea drugs.

Materials and methods: In 101 T2D patients (mean age 62 ± 10 years, 50% women, median T2D duration of 2.3 years) sulfonylurea drug was added to the antidiabetic treatment due to failure of metformin monotherapy (defined as HbA1c $> 7\%$). Fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c, DCCT standard) was measured at the baseline and after 6 months of combination therapy (metformin and sulfonylurea). Six gene polymorphisms were determined by real-time PCR with melting curve analysis (ABCC8 rs757110 [Ser1369Ala], KCNJ11 rs5219 [Glu23Lys], TCF7L2 rs7903146, KCNQ1 rs163184, MTNR1B rs10830963, GIPR rs10423928).

Results: Mean baseline HbA1c was $8.07 \pm 0.97\%$ and mean HbA1c reduction after 6 months of sulfonylurea treatment was $1.07 \pm 0.77\%$. We observed smaller HbA1c decrease in carriers of the risk minor allele of TCF7L2 rs7903146 C \rightarrow T ($p = 0.007$) and larger HbA1c decrease in carriers of the risk minor alleles of polymorphisms located in the genes encoding subunits of the ATP-dependent potassium channel KCNJ11 rs5219 G \rightarrow A ($p = 0.030$) a ABCC8 rs757110 T \rightarrow G ($p = 0.042$). Reduction in on-treatment FPG was significantly lower in homozygous carriers of the risk allele G of the rs163184 T \rightarrow G polymorphism compared with alternative genotypes in the gene encoding voltage-gated potassium channel (KCNQ1) ($p = 0.037$). After Bonferroni's correction for multiple testing (6 polymorphisms) we confirmed significant association only for the polymorphism TCF7L2 rs7903146 and HbA1c decrease after 6 months of sulfonylurea treatment. Mean HbA1c decrease across the TCF7L2 rs7903146 genotypes was as follows (mean \pm SEM): CC

$1.22 \pm 0.11\%$, CT $0.90 \pm 0.10\%$ a TT $0.85 \pm 0.31\%$. In the additive genetic model adjusted for age, sex, baseline HbA1c, body mass index and sulfonylurea dose we found significant per T allele difference in the reduction of HbA1c (-0.22% , $p = 0.007$; $p = 0.042$ after Bonferroni's correction).

Conclusion: Our results indicate lower efficacy of the treatment with sulfonylurea drugs in carriers of the risk T allele of TCF7L2 rs7903146 polymorphism. Further studies are needed to confirm our findings, to elucidate the pathogenetic mechanism as well as to further study its clinical implications.

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PS 003 Genetics and glucose homeostasis

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PPARGgamma Pro12Ala and ADAMTS9 rs4607103 are determinants of insulin sensitivity in both non-diabetic and type 2 diabetic Italian subjects

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Background and aims: The PPARGgamma Pro12Ala variant (rs1801282) is considered a determinant of type 2 diabetes risk at least in part through impaired insulin sensitivity and to play almost no role at all in Southern Mediterranean. The C allele of rs4607103 of a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 9 gene (ADAMTS9) is associated to type 2 diabetes risk and to reduced insulin sensitivity. However, these latter data were obtained in cohorts of nondiabetic individuals of North European ancestry. We investigated whether PPARGgamma rs1801282 and/or ADAMTS9 rs4607103 affect type 2 diabetes and insulin sensitivity in Italian subjects with normal and impaired glucose regulation (NGR and IGR respectively) and with newly diagnosed type 2 diabetes belonging to the GENFIEV Study and to the Verona Newly Diagnosed Type 2 Diabetes Study (VNDS), respectively. **Materials and methods:** In 621 GAD-negative, drug treatment naive patients (mean±SD; age 58.6±10 years, BMI 29.9±5 kg/m², HbA1c 7±1.3 %) from VNDS and in 674 subjects (334 NGR and 340 IGR) from GENFIEV (age 48.8±11.4 years, BMI 29±6 kg/m², HbA1c 5.6±0.5 %) we: 1. genotyped rs1801282 of PPARGgamma and rs4607103 of ADAMTS9; 2. assessed beta cell function by state-of-art mathematical modelling of glucose/C-peptide curves during the OGTT; 3. assessed insulin sensitivity, as M value during the euglycemic hyperinsulinemic clamp in VNDS (units: µmol·min⁻¹·m⁻² BSA) and as the HOMA insulin resistance score (HOMA-IR) in the GENFIEV.

Results: Both rs1801282 of PPARGgamma and rs4607103 of ADAMTS9 showed weak, inconsistent associations with beta cell function. Both C risk alleles of rs1801282 (under a recessive model) and rs4607103 were associated to type 2 diabetes (O.R.= 1.6 p<0.04 and O.R.= 1.39 p=0.03, respectively, after correcting for age, gender and BMI). The C allele of rs1801282 was associated to more severe insulin resistance in the entire GENFIEV dataset under a recessive model (HOMA-IR: +0.32±0.05, p<0.03), while the C allele of rs4607103 was associated to reduced insulin sensitivity only in the NGR subjects (HOMA-IR: +0.18±0.08, p<0.04). Both alleles were associated to reduced insulin sensitivity in VNDS (rs1801282 C: -90.0± 6.5, p<0.04, under a recessive model; rs4607103 C allele: -63.45±2.3, p<0.02), with mutual additive effects of each variant (p<0.05 by multivariate ANOVA). We then computed a score ranging from a minimum of 0 (double genotype rs1801282GG/GC-rs4607103TT) to a maximum of 3 (double genotype rs1801282CC-rs4607103), in order to analyse the combined effect of rs1801282 and rs4607103. The higher was the score, the lower was the insulin sensitivity (-69.4±2.4, p<0.005).

Conclusion: Both PPARGgamma and ADAMTS9 variants are associated to the presence of type 2 diabetes and exert an additive detrimental effect on insulin sensitivity both in nondiabetic individuals and in diabetic patients. Thus, in Italians they may be considered two prominent “insulin resistance genes” of type 2 diabetes.

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A genetic risk score for type 2 diabetes is also associated with a higher risk for impaired glucose tolerance among obese people

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Background and aims: Single nucleotide polymorphisms among approx. 40 genes have been associated with an increased risk for type 2 diabetes (T2D) in genome-wide association studies. It is not known whether a similar genetic impact on pre-diabetes (impaired glucose tolerance [IGT] or impaired fasting glycemia [IFG]) exists.

Materials and methods: In our cohort of 1442 non-diabetic subjects of European origin (normal glucose tolerance [NGT] n=1046, IFG n=142, IGT n=140, IFG+IGT n=114, reduced insulin secretion or reduced insulin sensitivity have been shown for 9 SNPs in previous studies. We analyzed these SNPs (within or in the vicinity of the genes TCF7L2, KCNJ11, HHEX, SLC30A8, WFS1, KCNQ1, MTNR1B, FTO, PPARG) for association with pre-diabetes.

Results: No association between the genetic risk load and the prevalence of IGT or IFG could be shown for the overall cohort. However, the risk score was significantly associated (p=0.005) with IGT or IGT+IFG for subjects with a body mass index over 30 kg/m². This association became significant for all subjects with pre-diabetes when only SNPs with a predominant effect on insulin secretion were taken into account.

Conclusion: We found an association of T2D genetic risk factors and IGT/IFG only in the obese. The absence of an influence of these genes on the transition from NGT to IGT in non-obese persons suggests that other (environmental) effects may be more important for this subgroup.

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Variants in CACNA1E affect beta cell function and glucose homeostasis in newly diagnosed type 2 diabetes patients

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Background and aims: The voltage-dependent Ca²⁺ channel CaV2.3 gene (CACNA1E) has been associated to risk of type 2 diabetes and impaired insulin secretion. We tested whether CACNA1E variability, assessed by genotyping 10 tag SNPs (rs558994, rs679931, rs2184945, rs10797728, rs3905011, rs12071300, rs175338, rs3753737, rs225338 and rs4652679), covering ~95% of common variability of CACNA1E genomic region, affects type 2 diabetes related phenotypes in patients with newly diagnosed type 2 diabetes.

Materials and methods: We assessed the metabolic phenotype of 595 consecutive GAD-negative, drug treatment naive patients (mean±SD; age: 58.5±10.2 yrs; BMI: 29.9±5 kg/m², HbA1c: 7.0±1.3) with newly diagnosed type 2 diabetes by state-of-art methods (mathematical modelling of glucose/C-peptide curves during an OGTT and euglycemic insulin clamp, for β-cell function and insulin sensitivity, respectively). For β-cell function we analyzed the stimulus response curve relating glycemia (selected values of 5.5, 8.0, 11.0, 15.0 and 20.0 mmol/l) to insulin secretion rate (units: pmol·min⁻¹·m⁻² BSA). Insulin sensitivity was assessed as M value during the clamp (units: µmol·min⁻¹·m⁻² BSA).

Results: Both major alleles of rs2184945 and rs3905011 associated to reduced beta cell function (rs2184945 A allele p<0.01 under a recessive model; rs3905011 G allele p<0.005). We then computed a score ranging from a minimum of 0 (double genotype rs2184945AA-rs3905011GG) to a maximum of 4 (double genotype rs2184945TT-rs3905011AA), in order to analyse the combined effect of rs2184945 and rs3905011. The lower was the CACNA1E score, the lower was the insulin secretion rate in response to glucose (p<0.005). Additional associations were found between rs2184945 and 2-hours plasma glucose (p<0.01 under a recessive model) and between rs3905011 and fasting plasma glucose (p<0.02), 2-hours plasma glucose (p<0.05) and HbA1c (p<0.03). Finally the minor allele A of rs225338 was associated to higher insulin sensitivity (p<0.05).

Conclusion: Our data suggest that in patients with newly diagnosed type 2 diabetes genotyping CACNA1E might be useful to infer the metabolic phenotype and to select a personalized treatment.

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Glucose-raising genetic variants in MADD and ADCY5 demonstrate effects on proinsulin-to-insulin conversion

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Background and aims: Recent meta-analyses of genome-wide association studies revealed new genetic loci associated with fasting glycemia. For most of these loci, the mechanism of action in glucose homeostasis is unclear. Es-

tabulating metabolic phenotypes for these genetic variants can deliver clues to their pathomechanism.

Materials and methods: We genotyped our cohort of 1782 non-diabetic subjects for 12 single nucleotide polymorphisms in or near the genes GCK (rs4607517), DGKB (rs2191349), GCKR (rs780094), ADCY5 (rs11708067), MADD (rs7944584), ADRA2A (rs10885122), FADS1 (rs174550), CRY2 (rs11605924), SLC2A2 (rs11920090), PROX1 (rs340874), GLIS3 (rs7034200) and C2CD4B (rs11071657). Insulin, C-peptide and proinsulin were measured at 5 points during an oral glucose tolerance test (OGTT). Parameters of insulin secretion (AUC Insulin0-30/AUC Glucose0-30, AUC C-peptide0-120/AUC Glucose0-120), proinsulin-to-insulin conversion (fasting proinsulin, fasting proinsulin/insulin, AUC Proinsulin0-120/AUC Insulin0-120) and insulin resistance (HOMA-IR, Matsuda-Index) were calculated.

Results: After adjustment for confounding variables, the effect alleles of the ADCY5 and MADD SNPs were associated with proinsulin-to-insulin conversion ($p=0.002$ and $p=0.0001$, respectively). GLIS3 was nominally associated with proinsulin-to-insulin conversion and insulin secretion. DGKB and PROX1 were nominally associated with insulin secretion. Nominally significant effects on insulin sensitivity could be found for MADD and PROX1.

Conclusion: We confirmed previous findings on the role of a genetic variant in MADD on proinsulin-to-insulin conversion. By examining parameters of glucose-stimulated proinsulin-to-insulin conversion during an OGTT, we additionally established that the SNP in ADCY5 is also implicated in defective proinsulin-to-insulin conversion. These effects may also be related to neighboring regions of the genome.

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The rs10195252 near the growth factor receptor-bound protein 14 (GRB14) gene is associated with diabetes risk and plasma triglycerides in a Mediterranean population

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Background and aims: The Grb proteins (growth factor receptor-bound proteins) constitute a family of structurally related multidomain adapters with diverse cellular functions. Grb14, in particular, has been implicated in the regulation of insulin receptor signalling. In animal models, ablation of Grb14 enhances insulin action in liver and skeletal muscle and improves whole-body tolerance. Elevated expression of Grb14 may contribute to states of insulin resistance. Taken together these results are consistent with a role of Grb14 in the regulation of insulin sensitivity and the development of insulin resistance in humans. However, there are not studies in humans investigating the association between GRB14 gene variation and diabetes. Our aim was to study the association between the rs10195252 polymorphism near the GRB14 gene and diabetes related variables in a high cardiovascular risk Mediterranean population.

Materials and methods: We studied 1050 high cardiovascular risk (average age: 67±6 years) subjects with either diabetes, or three or more cardiovascular risk factors participating in the PREDIMED (PREvención Dieta MEDiterránea) Study, recruited in the Valencia region, Spain. Anthropometric measures were directly measured by standard methods. Fasting glucose was determined. Type 2 diabetes was assessed by standard criteria and the rs10195252 was analyzed. Diet and other lifestyle variables were assessed by validated questionnaires.

Results: Genotype frequencies for rs10195252 polymorphism were 35% CC, 50% CT and 15% TT in the population as a whole. Prevalence of diabetes was 46%. We found a significant association between the rs10195252 polymorphism and diabetes. This inverse association was significant ($P=0.021$) when we considered an additive model. However, we obtained a higher significant association when a recessive model was considered. Thus, homozygous subjects for the variant allele (TT) have a reduced prevalence of diabetes as compared with the other genotypes (35% vs 65%; $P=0.003$). After adjustment for gender, age and body mass index (BMI) the lower risk associated with the variant allele did not change (OR: 0.56; 95% CI: 0.38–0.83). Moreover, although in the whole population we did not find a significant association of this polymorphism with plasma lipid, we detected a significant interaction between this polymorphism and diabetes in determining triglyceride con-

centrations. The variant allele was significantly associated with lower triglyceride concentrations in non-diabetics. In diabetic subjects an opposite trend was found.

Conclusion: We reported for the first time a significant inverse association between the rs10195252 polymorphism near the GRB14 gene and diabetes risk in an older Mediterranean population, supporting a relevant role of the GRB14 gene in insulin sensitivity in humans.

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The melatonin receptor 1B gene (MTNR1B) in the self-contained population of Sorbs from Germany: association and evolutionary studies
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Background and aims: Several single nucleotide polymorphisms (SNPs) of the melatonin receptor 1B gene (MTNR1B) have been shown to be associated with elevated fasting plasma glucose (FPG) and impaired early insulin release. We therefore initiated this study to assess effects of MTNR1B variants on traits related to type 2 diabetes mellitus (T2DM) in the self-contained population of Sorbs from Germany. Furthermore, since comprehensive studies concerning the conservation of MTNR1B are lacking, we investigated natural selection patterns in vertebrates and human populations at this locus.

Material and methods: Five SNPs (rs10830962, rs4753426, rs12804291, rs7951037 and rs10830963) representing all linkage disequilibrium groups in MTNR1B as well as 5 kb of the 5'- and 3.5 kb of the 3'-flanking region were genotyped in about 1000 subjects for subsequent association analyses on metabolic traits related to T2DM. All subjects are part of a cohort from an extensively phenotyped self-contained ethnic group in Eastern Germany, the Sorbs. Signatures of selection between species were investigated with phylogenetic analysis by maximum likelihood (PAML, $\omega = d_n/d_s$) and various tests of population genetic measures (e.g. the fixation index (Fst), Tajima's D) were performed.

Results: We were able to replicate previously known associations between MTNR1B SNPs and glucose parameters like FPG (rs10830963, adjusted $P < 0.001$) as well as insulin measurements (fasting plasma insulin: rs4753426 and rs10830963, adjusted $P < 0.05$, 30 min insulin: rs4753426 and rs10830963, adjusted $P < 0.05$). Also an impact on homeostasis model assessment of beta-cell function (rs4753426 and rs10830963, adjusted $P < 0.01$) could be shown. PAML-analyses revealed that MTNR1B was strongly conserved between species ($\omega = 0.2583$). Structures important for the receptor function are also conserved. On the lineage leading to human adaptive selection/few conservation was observed ($\omega = 1.1030$). Population genetic measures further indicated natural selection.

Conclusion: Our data support the physiologic relevance of MTNR1B in the context of glucose and insulin homeostasis and imply recent selection at this locus. The exact mechanisms linking this possible adaptation of circadian rhythm to alterations in glucose metabolism has to be further illuminated.

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Common variation in lanosterol synthase gene predispose to type 2 diabetes

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Background and aims: The aim of this study is to find causative genes/SNPs for T2DM in Japanese under the peak at 21q22.3 identified by genome-wide linkage analysis (affected sib-pair analysis), where the baseline LOD score of 1.92 increased to 2.42 and 2.59 when we used the leanest 116 families (BMI <24); and 146 families with age at diagnosis under 56 years, respectively.

Materials and methods: Capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) analysis was performed for screening of

causative genes under the concerning region using 96 patients from original linkage study and 200 controls. The positive association was replicated using following sample sets (Japan: 779 cases / 1500 controls, Korea: 756 cases / 628 controls, Denmark: 3,531 cases / 4,885 controls, and U.K. 1,999 cases / 1,502 controls from WTCCC genotype data).

Results: We identified LSS (lanosterol synthase, 2,3-oxidosqualene cyclase) showed a significant association with T2DM in Japanese (rs2075906 at intron 17, $P = 3.2 \times 10^{-4}$, OR = 1.25). The association was further replicated in Korean and Europeans (overall $P = 2.0 \times 10^{-6}$, OR = 1.16). We found that rs2075906 variant matches to Yin Yang 1, the motif which binds to LSS promoter and represses the synthesis of LSS. The rs2075906 risk genotype (CT+TT) was associated with the expression level of LSS ($P = 0.029$) and cholesterol indicators in healthy subjects ($P < 0.05$). HOMA-IR of cases with risk genotype was significantly higher than those with non risk genotype ($P = 0.029$), while no association was observed in HOMA-beta in T2DM.

Conclusion: We found that LSS, a cholesterol biosynthesis enzyme, is a susceptible gene to T2DM in Asian and European populations through cholesterol metabolism and insulin sensitivity.

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Association of rs2241766 and rs 1501299 ADIPOQ polymorphisms with coronary heart disease in Chinese Han population: a meta-analysis

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Background: Adiponectin is an important adipokine with insulin-enhance and anti-inflammatory action. The ADIPOQ rs2241766 and rs1501299 polymorphisms have been examined for association with coronary heart disease (CHD) over the past decade, but results remained inconsistent.

Aim: The meta-analysis of studies based on Chinese Han population to assess the association between the ADIPOQ rs2241766 and rs1501299 polymorphisms and the risk of CHD.

Data sources: A systematically search of the Chinese VIP, Chinese National Knowledge Infrastructure (CNKI) and Chinese Wang Fang database, and articles listed in reference lists of key article. No language restrictions.

Study selection: Studies were included if they had data on the ADIPOQ rs2241766 and rs1501299 polymorphisms genotype or alleles frequency and involved CHD as an outcome. Total 10 studies met these criteria.

Data extraction: Data were collected independently by two reviewers on ADIPOQ rs2241766 and rs1501299 genotype, alleles frequency and case-control status.

Results: For rs2241766, there was a trend toward increase CHD risk comparing variant genotype with wild-type genotype in Chinese Han population, the pooled OR was 1.07 (95% CI 0.81–1.42); however, in Chinese western region subgroup, individuals with variant genotype had 47% higher risk for CHD than those with wild-type genotype (OR, 1.47; 95% CI 1.08–2.00). For rs1501299, individuals with variant genotype had a significantly lower risk to CHD in comparison with individuals with wild-type genotype, the pooled OR was 0.86 (95% CI 0.68–1.08).

Conclusion: The rs1501299 polymorphism is found to be strongly associated with CHD risk, and wild-type genotype is susceptible to CHD in Chinese Han population. And the result of our meta-analysis supports a modest role for rs2241766 ADIPOQ polymorphisms in CHD risk, only in western region population, individuals with variant genotype are more susceptible to CHD.

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Effect of the GRM8 Variant rs2237781 on eating behaviour in the self-contained population of Sorbs

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Background and aims: The glutamate receptor, metabotropic 8 (GRM8) on chromosome 7q31 belongs to the group III of the G-protein-coupled glutamate receptors which are linked to the inhibition of the cyclic AMP cascade and regulate the pre-synaptic glutamate release. Data from animal studies in-

dicate that GRM8 may be involved in the regulation of the neuropeptide Y (NPY) and melanocortin pathway and thereby plays an integral role in food intake and metabolism via the hypothalamic pathway. Therefore, we studied effects of genetic variants in the GRM8 on eating behaviour assessed by the German version of the three factor eating behaviour questionnaire.

Materials and methods: All subjects are part of a self-contained population in Eastern Germany, the Sorbs. A total of 582 individuals (354 females, 228 males with mean age 47 ± 16 years and mean BMI 26.4 ± 4.7 kg/m²) extensively phenotyped for metabolic traits completed the revised version of the three-factor eating questionnaire. Genotyping of rs2237781 representing a linkage disequilibrium group within the GRM8 locus ($r^2 \geq 0.8$ and minor allele frequency ≥ 0.05) was performed using the TaqMan SNP Genotyping assay (Applied Biosystems, Inc., Foster City, CA). Genetic associations with restraint, disinhibition and hunger were assessed in an additive logistic regression model using age, gender and BMI as covariates. In addition, we performed further analyses stratified by gender. P-values < 0.05 were considered to provide nominal evidence for association.

Results: The minor A allele of variant rs2237781 (MAF 0.06) located in intron 2 of GRM8 is significantly associated with diminished restraint in eating behaviour (adjusted $P = 1.2 \times 10^{-4}$). Gender stratified analyses revealed stronger association in women (adjusted $P = 7.2 \times 10^{-4}$) compared to men (adjusted $P = 0.05$). Moreover, for disinhibition the association has been found in males only (adjusted $P = 7.0 \times 10^{-4}$). No association could be identified for intensity of hunger.

Conclusion: We propose GRM8 to be a candidate gene involved in eating behaviour. Further replication studies in independent cohorts and analyses of central effects of GRM8 in functional magnet resonance imaging (MRI)-studies are currently ongoing to elucidate the role of genetic variation in GRM8 in the control of eating behaviour.

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Microvascular dysfunction increases the risk of type 2 diabetes mellitus: a meta-analysis

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Background and aims: Type 2 diabetes mellitus (DM2) is characterized by microvascular dysfunction. Recent data support the hypothesis that impairment of microvascular function may cause insulin resistance and thus contributes to the development of DM2. The aim of this meta-analysis is to investigate whether microvascular dysfunction contributes to the development of impaired glucose tolerance and DM2.

Materials and methods: We searched MEDLINE and EMBASE for studies published from 1989 to April 15th 2010. Prospective studies were included if they focused on microvascular measurements (retinal diameters, skin microvascular endothelium-dependent and -independent reactivity, capillary density, peripheral vascular reactivity, microalbuminuria, or plasma biomarkers of microvascular endothelial dysfunction) in a population-based sample of individuals without DM2 at baseline. We conducted a meta-analysis by use of RevMan5 to determine the associations of microvascular function with glucose tolerance status, using the generic inverse variance method. The pooled relative risk and 95%CI of the fully adjusted models were estimated by use of the random effects model.

Results: Twenty-three studies met our pre-specified inclusion criteria. One standard deviation (1SD) increase in retinal venular diameter was associated with a 15% higher risk of impaired fasting glucose (IFG) (95%CI [1.01-1.31], $p=0.03$), and 1SD decrease in retinal arteriolar/venular-ratio (AVratio) was associated with a 14% higher IFG risk (95%CI [0.98-1.32], $p=0.08$). Arteriolar diameter was not associated with the development of IFG ($p=0.84$). With respect to DM2, 1SD decrease in AVratio was associated with a 18% higher DM2 risk (95%CI [1.08-1.29], $p<0.001$), whereas increased venular diameters and decreased arteriolar diameters were not significantly associated ($p=0.21$ and $p=0.21$ respectively). For endothelial dysfunction, 1SD increase in microalbuminuria was associated with a 53% higher DM2 risk (95%CI [1.25-1.88], $p<0.001$), while 1SD increase in plasma levels of von Willebrand Factor (vWF) and soluble inter-cellular adhesion molecule-1 (ICAM-1) were associated with a 33% (95%CI [1.03-1.72], $p=0.03$) and 22% (95%CI [1.08-1.38], $p=0.002$) higher risk of DM2 respectively.

Conclusion: These data indicate that microvascular dysfunction, and in particular endothelial dysfunction, is associated with a higher risk of IFG and DM2. This suggests a role for microvascular dysfunction in the (early) pathogenesis of DM2.

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Non-diabetic hyperglycaemia as a risk factor of gallstones

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Background and aims: Recent epidemiological evidence has suggested that patients with diabetes mellitus are associated with an increased risk of gallstones. However, an association between non-diabetic hyperglycemia and a risk of gallstones has not been thoroughly investigated at all or has been examined with a simple binary category (pre-diabetic or not). In the present study, we hypothesized that there could be a continuous association between non-diabetic hyperglycemia and a risk of gallstones and examined the hypothesis.

Materials and methods: We conducted a cross-sectional study in the medical check-up unit of one general hospital. All participants ($n=2,761$) who underwent a medical check-up focusing on metabolic syndrome from May 2008 to December 2010 were invited to the study. Blood samples and abdominal ultrasound images were obtained after an overnight fast. Those with known diabetes or those whose fasting plasma glucose (FPG) levels were more than or equal to 7.0 mmol/l were excluded ($n=439$). Ninety-two participants who had undergone cholecystectomy were also excluded. Data were not available among 8 subjects. Finally, a total of 2,222 participants were included in the analysis. A diagnosis of gallstones was confirmed by the ultrasound imaging.

Unconditional logistic regression was performed to examine the association between FPG levels and a risk of gallstones, having adjusted for possible confounders.

Results: Gallstones were identified among 212 participants (9.5%) but not among 2010 participants (90.4%). FPG levels were 5.58 ± 0.54 mmol/l among those with gallstones while 5.41 ± 0.52 mmol/l among those without ($p<0.001$). After adjustment for age, gender, BMI, WHR, systolic BP, diastolic BP, HDL-cholesterol levels, TG levels, smoking status and alcohol intake, a positive association between FPG levels and a risk of gallstones were found. Compared with individuals with an FPG of <5.0 mmol/l, the ORs of gallstones for those with an FPG of 5.0 to <5.5 mmol/l, those with an FPG of 5.5 to <6.0 mmol/l, those with an FPG of 6.0 to <6.5 mmol/l and those with an FPG of 6.5 to <7.0 mmol/l were 1.20 (95% CI = 0.73-1.98), 1.39 (95% CI = 0.83-2.32), 1.47 (95% CI = 0.82-2.63) and 2.31 (95% CI = 1.09-4.92), respectively (P for trend = 0.030). FPG levels were also analysed as a continuous variable, yielding a significant OR of 1.35 (95% CI = 1.01-1.81) for a 1 mmol/l increment in FPG levels.

Conclusion: The present study found that FPG levels were positively and continuously associated with an increased risk of gallstones among non-diabetic individuals. This association remained significant even after adjustment for possible confounders including BMI and WHR, suggesting that FPG levels could be associated with the formation of gallstones independently of obesity or other factors related to metabolic syndrome, which have been proposed as risks of gallstones. However, the causal association cannot be demonstrated in the present study because of the cross-sectional study design. Further studies are required in this area.

ORs of gallstones according to FPG levels

	Gallstones				Adjusted OR	95% CI	P for trend
	No n=2010	%	Yes n=212	%			
FPG (categorical)							0.030
FPG<5.0	394	19.6	23	10.9	1.00		
5.0≤FPG<5.5	759	37.8	70	33.0	1.20	0.73-1.98	
5.5≤FPG<6.0	565	28.1	70	33.0	1.39	0.83-2.32	
6.0≤FPG<6.5	236	11.7	35	16.5	1.47	0.82-2.63	
6.5≤FPG<7.0	56	2.8	14	6.6	2.31	1.09-4.92	
FPG (continuous)							
an increment in 1mmol/l					1.35	1.01-1.81	

ORs were adjusted for age, gender, BMI, WHR, systolic BP, diastolic BP, TG, HDL-cholesterol, smoking status and alcohol intake.

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Analysis of hospital admissions for new-onset diabetes of the adult in the period 2003-2010 in a Mediterranean area: impact and characteristics of the non-Caucasian population

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Background and aims: Percentage of non-Caucasian population is increasing in Catalonia and particularly in Barcelona metropolitan area due to the growing of immigration in recent years. In other well-developed world areas with high percentage of non-Caucasian population like North-America, symptomatic diabetes onset in adults has differential characteristics in non-Caucasian compared with Caucasian patients, particularly in terms of autoimmunity and beta-cell function. Little is known about characteristics of symptomatic diabetes onset in non-Caucasian adult population in Mediterranean European countries. The aim was to evaluate the percentage of non-Caucasian patients in our unit admissions for new-onset symptomatic diabetes of the adult in the period 2003-2010 and to compare them with Caucasian subjects with regard to clinical, biochemical, immunological, and beta-cell function characteristics.

Materials and methods: Our study was a retrospective analysis. Patients admitted in our unit with the diagnosis of new-onset symptomatic diabetes in the period 2003-2010 were included if they were 18-40 years old and had no concomitant relevant illnesses. Annual prevalence of non-Caucasian popula-

tion was determined. Data from non-Caucasian patients were compared with those of Caucasian subjects in terms of clinical (gender, age, symptoms duration, weight loss, BMI, insulin dose at discharge, severity of metabolic situation), biochemical (glycemia, HbA_{1c}, pH, bicarbonate), immunological (GAD and IA-2 autoantibodies), and beta-cell function (glucagon test) characteristics. Groups were compared with parametric tests; statistical significance was defined as $p < 0.05$.

Results: 1052 patients were admitted in our unit in the period 2003–2010 with diabetes as the main diagnosis. Of those, 311 were diagnosed as new-onset diabetes, and 197 were 18–40 years old. Data with regard to autoimmunity and beta-cell function were available in 168 (85.3%), 136 Caucasians and 32 non-Caucasians. The global prevalence of non-Caucasians patients (mainly from Maghrib and South-America) was 23.9%, with progressive increase in recent years. Groups were comparable in terms of age (28.7 ± 5.8 vs. 30.5 ± 6.0 years, Caucasians and non-Caucasians respectively), BMI (23.4 ± 5.0 vs. 25.1 ± 5.1 kg/m²), duration of symptoms (7.8 ± 6.7 vs. 6.7 ± 5.9 weeks) and weight loss (8.2 ± 5.4 vs. 9.2 ± 4.8 kg). In contrast, differences between groups were significant in terms of pH (7.34 ± 0.11 vs. 7.38 ± 0.52 Caucasians and non-Caucasians respectively, $p < 0.01$), bicarbonate (23.7 ± 6.9 vs. 26.1 ± 3.9 mmol/L, $p < 0.05$), percentage of ketoacidosis (14.7% vs. 3.2%, $p < 0.05$), presence of autoantibodies (73.5% vs. 28.1%, $p < 0.01$) and stimulated C-peptide (0.42 ± 0.39 nmol/L vs. 0.70 ± 0.56 nmol/L, $p < 0.05$). The remaining clinical and biochemical parameters were comparable between groups.

Conclusion: The number and proportion of non-Caucasian patients has significantly increased in our hospital admissions for new-onset diabetes in adult population. These patients present with a lower presence of autoimmunity data and better preserved beta-cell function than Caucasian population. Evolution analyses are needed to clarify the pathological mechanisms involved.

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STARDUST - study of the treatment and prevalence of renal disease in UK diabetes mellitus type 2 patients

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Background and aims: Kidney complications are common in patients with type 2 diabetes mellitus (T2DM). Treating T2DM patients with kidney impairment can pose a challenge for clinicians as many glucose lowering treatments are contraindicated in moderate and severe kidney impairment. This study aims to investigate antidiabetic treatments and insulin prescribed to T2DM patients with and without kidney impairment.

Materials and methods: The study was based on the UK General Practice Research Database (GPRD). Patients with eGFR < 60 mL/min/1.73m² were defined as having renal impairment, patients with eGFR > 60 mL/min/1.73m² were used for comparison. The nested case-control design was used to ensure both groups were comparable in terms of age, gender distribution and diabetes duration. Patients were included in the study if they had a diagnosis of type 2 diabetes and were 40 years or older at the time of diagnosis. Patients also had to have at least 12 months follow-up after the diagnosis and at least 1 record of serum creatinine or a diagnostic code of Chronic Kidney Disease (CKD) stage. Outcomes were category of glucose lowering therapies prescribed to patients with and without renal impairment.

Results: 168,265 patients met the inclusion/exclusion criteria, 75.54% of them had eGFR > 60 mL/min/1.73m², 24.46% had eGFR < 60 mL/min/1.73m². 9678 patients with renal impairment were matched with 59,168 patients without renal impairment. The average age was 75 and mean diabetes duration of 8.35 years. Nearly 80% of T2DM patients with renal impairment were diagnosed with hypertension compared to 37% of patients without renal impairment. Stroke, chronic obstructive pulmonary disease, coronary heart disease and hyperlipidaemia were also twice as common in patients with renal impairment compared to controls. Thirty-one per cent of patients without renal impairment were prescribed metformin compared to 48% ($P < 0.0001$) of patients with renal impairment. Amongst patients with renal impairment, 23% of patients with eGFR between 30 and 45 mL/min/1.73m² and 31.78% of patients with eGFR < 30 mL/min/1.73m² were prescribed with metformin. Number of prescriptions per patient was also higher in patients with renal impairment. On average, patients with renal impairment received 31 metformin prescriptions per person compared to 15 in patients without renal impairment. Prescriptions of sulphonylurea were also more common in patients with renal impairment, 34% versus 17% ($p < 0.0001$). However, the difference

was greatest for prescriptions of insulin, 8% of patients without renal impairment versus 22% ($p < 0.0001$).

Conclusion: Renal impairment is common in patients with T2DM. Patients with renal impairment were more likely to have comorbidities such as hypertension than patients without renal impairment. Treatments of T2DM were also very different amongst patients with and without renal impairment. Patients with renal impairment were more likely to receive prescriptions of glucose lowering treatments and were also receiving more prescriptions compared to the controls. In contradiction with the NICE guideline on type 2 diabetes, metformin remains to be commonly used in patients with eGFR < 30 mL/min/1.73m². Given similar duration of diabetes, prescriptions of insulin were two and half times as common in patients with renal impairment compared to patients without renal impairment. This may indicate a lack of choice in glucose lowering treatment for T2DM patients with renal impairment

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The relationship between HbA_{1c} variability for 10 years and beta cell function and diabetic nephropathy in new onset type 2 diabetes

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Background and aims: A1c is the important parameter for average glucose control but A1c may be fluctuating for long time and its degree can be so different in each diabetes patient. Insulin secretory function of type 2 diabetes has been known to be progressively deteriorated after diagnosed. The clinical meanings and the significance of the A1c variability on the chronic diabetic complications are still uncertain. We performed this study to see the A1c variability during the first 10 years after diagnosis of type 2 diabetes, and its relationship with beta cell function and diabetic nephropathy in clinical practice.

Materials and methods: Data were from 518 newly onset diabetes. Their mean age was 53.0 ± 12.2 years and the percent of male and female was 46.5% and 53.2%, respectively. A1cs, FBS, c-peptide, insulin levels, urine profiles and s-Cr were collected serially at least once in every year for 10 years since diagnosed as diabetes. Intrapersonal SD of serially measures A1c was considered a measure of variability of A1c.

Results: When the patients were divided by SD of A1c tertile, lowest SD group showed older age at diagnosis, lower HbA_{1c} at diagnosis and after 1-year and lower mean HbA_{1c} during the subsequent 9 years. Highest SD group showed lower fasting baseline c-peptide and HOMA- β values compared to lowest SD group, but there were no differences in HOMA-IR. The patients in highest SD group had higher decrease in c-peptide and HOMA- β during 10 years. Among the groups divided by diabetic nephropathy, normal group showed significantly lower mean A1c and mean SD of A1c compared with the group having overt proteinuria and abnormal serum creatinine values.

Conclusion: HbA_{1c} variability in newly diagnosed type 2 diabetes is inversely related with the marker of β -cell function at the time of diagnosis, and might have correlation with the development of diabetic nephropathy.

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Trends in hospital admissions for diabetes and diabetes-related lower extremity amputations in the Republic of Ireland over a five year period

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Background and aims: Diabetes is a common chronic disease in the Republic of Ireland and the prevalence is rising. The WHO Global Burden of Disease study estimated a prevalence of 86,000 cases of diabetes in the Republic of Ireland in 2000, with a projected increase to 157,000 cases in 2030. Diabetes has many complications including Lower Extremity Amputation; a significant complication that is costly to the individual and the state. Lower extremity amputations are classified as major or minor based on anatomical location. Site of amputation affects quality of life and future functional capacity. The aims of this study are to identify in-patient hospital admissions of diabetic patients for any cause and for Lower Extremity Amputations (Total, Major and Minor) and to examine trends in admissions over a 5 year period. This is the first national estimate of diabetes-related Lower Extremity Amputation in the Republic of Ireland.

Materials and methods: This study is a retrospective review of hospital admissions using Hospital In-Patient Enquiry (HIPE) data, which includes major diagnoses and procedures performed during hospital admission. All in-patient admissions for which a diabetes diagnosis (ICD Codes E10-14) was recorded were extracted from the HIPE system for years 2005-2009 inclusive. The subgroup of these patients admitted for Lower Extremity Amputation was identified (ICD Codes 1484, 1505, 1533). Age standardised rates were calculated for each year and trend analysis carried out using Stats Direct.

Results: There were 283,332 hospital admissions pertaining to 122,714 patients with a diagnosis of diabetes over the 5 years. The direct standardised rates of hospital admissions in the diabetic population increased over time from 861/100,000 to 1,569/100,000 general population ($p=0.02$). From 2005-2009, there were 1,622 hospital admissions for diabetic patients receiving amputations. This involved 1,358 patients. The direct standardised rates of hospital admission for diabetes-related Lower Extremity Amputation increased from 7.5/100,000 to 8.7/100,000 general population over this period ($p=0.03$). The increase in amputations was mostly due to an increase in minor amputation rates.

Conclusion: This study found that as the prevalence of diabetes in the Republic of Ireland has increased over time, the number and rate of hospital admissions for diabetic patients and diabetes-related Lower Extremity Amputations has also increased. Fortunately, the rise in amputation rates is in line with the increase in diabetes prevalence and not in excess of it. Furthermore, while amputation rates have increased overall, major amputation rates have remained static and only minor amputation rates have increased. Minor amputations are not as clearly indicative of poor quality of care as major amputations. Thus, intervention strategies to improve foot-care may underlie the trends observed in this study. Improving diabetes care needs to continue to be prioritised in the Republic of Ireland to further reduce complication rates. Supported by: Health Research Board, Ireland

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Type 2 diabetes and hypoglycaemia in the elderly: insights from the 6-month prospective DiaRegis follow-up

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Purpose: Intensified glucose control in the elderly has been questioned because of complications associated with pharmacotherapy such as hypoglycaemia. **Methods:** Prospective registry of pts with type-2 diabetes and failure of oral mono- or dual antidiabetic therapy. Comparison of patients at an age of < 60 years ($n=1268$) with those ≥ 70 years ($n=1397$).

Results: Elderly patients had a substantially later diabetes onset and a better glucose control than the young (HbA1c 7.3 vs. 7.6%; $p<0.0001$; fasting blood glucose 138 vs. 146 mg/dl; $p<0.0001$; postprandial glucose 180 vs. 189 mg/dl; $p<0.0001$). Furthermore, cardiovascular disease was more frequent (CAD, stroke/TIA, heart failure). Elderly patients' cardiovascular pharmacotherapy was more intense and there was a higher use of sulfonylureas (SU, 34.8 vs. 22.0%; $p<0.0001$), while metformin and newer oral drugs (glitazones, DPP-4-inhibitors) were used less frequently. Any hypoglycaemia was a frequent complication in the elderly within 12 months prior to enrolment (12.7 vs. 9.1%; $p<0.01$). This led investigators to dismiss SUs in elderly patients with anamnestic hypoglycaemia (48.0% prior vs. 18.6% after therapy change). The use of DPP-4-inhibitors increased (5.1% prior vs. 30.5% at baseline) and insulin was started in 22.6%. Overall, therapy change led to improved HbA1c in both groups (6.9% in elderly and younger patients) at 6 months. In elderly patients with anamnestic hypoglycaemia HbA1c was only slightly reduced from 7.0 to 6.9% and 29% suffered from incident hypoglycaemic events vs. 5.9% in elderly patients without hypoglycaemia. Incident unstable angina, autonomous and peripheral neuropathy, non-proliferative retinopathy and clinically relevant depression were more frequent in elderly patients with anamnestic hypoglycaemia than in those without.

Conclusion: Elderly patients had a distinct risk profile and higher incidence of hypoglycaemia which may reasonably be attributed to the increased use of sulfonylureas. This was associated with a higher risk for incident morbidity in the following 6 months.

	Age < 60 years	Age ≥ 70 years	p-value
Coronary artery disease (%)	8.4	28.0	<0.0001
Post MI (%)	49.5	28.5	<0.0001
Stable angina (%)	19.8	33.7	<0.0001
Stroke / TIA (%)	2.5	6.8	<0.0001
Heart failure (%)	2.6	18.7	<0.0001
Incident events (6 months)	Age ≥ 70 years with anamnestic hypo	Age ≥ 70 years without anamnestic hypo	
Unstable angina (%)	2.4	0.6	<0.05
Autonomous neuropathy (%)	4.1	1.3	<0.01
Peripheral neuropathy (%)	10.7	4.7	<0.01
Non-proliferative retinopathy (%)	4.1	1.1	<0.01
Hypoglycaemia (%)	29.0	5.9	<0.0001

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Diabetes, co-morbidities and long-term mortality after discharge from geriatric inpatient care

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Background: Many studies have demonstrated that diabetes is associated with an increase in both cardio-vascular (CV) and all cause mortality, even in older subjects. Patients in medical or geriatric care represent a particular population with a high prevalence of co-morbidities and functional disabilities. We have previously shown that diabetes in such patients is generally characterized by unexpectedly good glycemic control, albeit in the context of malnutrition and/or co-morbidities. It is unclear whether diabetes per se remains associated with a high prevalence of co-morbidities and long-term mortality in such a population.

Methods: We determined the prevalence of co-morbidities and functional disabilities according to diabetes status in a cohort of 444 patients (mean age 85.3 ± 6.7 ; 74.0% women) discharged from our geriatric service. Besides individual diseases, a global co-morbidity score (Chronic Illness Rating Scale, CIRS) was used. Functional disabilities were assessed using basic and instrumental activities of daily living (ADL and IADL). We further analysed the impact of diabetes, co-morbidities and functional disabilities on 4-year mortality using simple and multiple Cox proportional hazard models.

Results: Diabetic patients had a higher BMI (23.4 ± 4.7 versus 27.1 ± 4.9 kg/m² in controls, $p<0.001$) and a higher prevalence of hypertension (81.9 v. 65.1%, $p=0.003$) and ischemic heart disease (33.7 v. 22.2%, $p=0.033$), but there was no difference in the prevalence of stroke or renal insufficiency. They had a higher prevalence of co-morbidities according to the CIRS score (15.1 ± 4.5 v. 13.8 ± 4.8 , $p=0.016$). Diabetic patients also had more functional disabilities as evidenced by lower ADL and IADL scores. However, there were no significant differences in the prevalence of cognitive impairment and depression. In simple Cox models a low BMI, the CIRS score, low serum albumin and functional disabilities were associated with all-cause 4-year mortality. Diabetes was also associated with mortality (Hazard Ratio 1.42, 95%CI 1.02-1.99, $p=0.041$), but only after adjustment for age, sex and BMI (or obesity status). This association persisted after further adjustment for hypertension, ischemic heart disease, stroke, atrial fibrillation and renal insufficiency. However, it disappeared after adjustment for the CIRS score or ADL and IADL scores.

Conclusions: In older patients discharged from geriatric care, diabetes is associated with a high prevalence of co-morbidities and functional disabilities. It is also associated with 4-year mortality, but only after adjustment for the inverse association with the BMI. Diabetes-associated mortality is accounted for by global co-morbidities and functional disabilities, rather than by individual vascular co-morbidities. They imply that the active care of all - rather than selected - co-morbidities is the key to improving the prognosis of older diabetic patients.

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The impact of treatment non-compliance on mortality in people with type 2 diabetes

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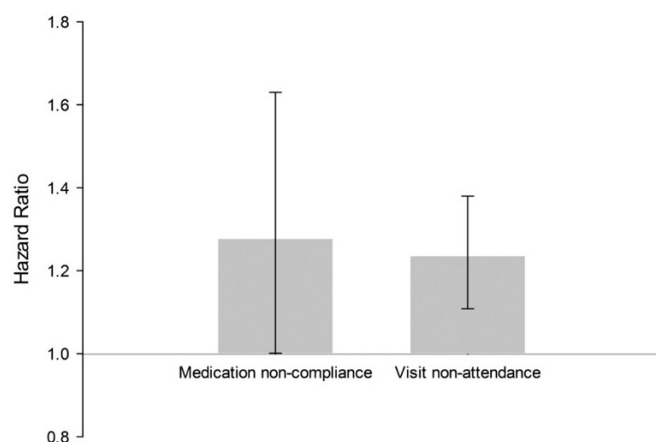
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Background and aims: Poor compliance with scheduled appointments for diabetes-related monitoring and treatment has been linked to poor glucose control. The aim of this study was to determine if a diagnostic record of poor medication compliance and/or non-attendance at medical appointments was associated with all-cause mortality in insulin treated people with type 2 diabetes.

Materials and methods: Data were extracted from The Health Improvement Network (THIN) research database comprising data on patients served by over 350 primary care practices in the UK. Patients were included in the study if they had type 2 diabetes or they had previously received at least one prescription for an oral antidiabetic agent, and then initiated insulin therapy. Diagnostic codes indicating non-compliance with medication or non-attendance at medical appointments were assessed during a 30-month period prior to baseline. Relative survival was compared by determining the adjusted progression to all-cause mortality using Cox proportional hazard models.

Results: A total of 15,984 insulin-treated patients with type 2 diabetes were identified for inclusion into the study (age 64 ± 13 years, 54% male, BMI 31 ± 6 , HbA1c $8.3 \pm 1.6\%$). The number of patients receiving a specific medical diagnosis of non-compliance or missing at least one scheduled clinic appointment during the assessment period was 705 (4.4%) and 6,227 (39.0%), respectively. Non-attendance was higher among those who were non-compliant with medication (odds ratio [OR] = 2.45; 95% CI 2.10–2.86). Following adjustment for age, gender, smoking status, HbA1c, BMI, systolic blood pressure, renal disease and the number of previous contacts with the primary care practitioner in the preceding 12 months, both the diagnosis of medication non-compliance (HR=1.28; 95% CI 1.00–1.63) and visit non-attendance (HR=1.24; 95% CI 1.11–1.38) were found to be independently associated risk factors for all-cause mortality.

Conclusion: Medication non-compliance and visit non-attendance - as assessed during routine care by primary care physicians - were independently associated with increased all-cause mortality in patients with type 2 diabetes receiving insulin. The small proportion of patients with a diagnostic code of medication non-compliance is likely to reflect the most obvious or severe cases. A more systematic investigation of the effects of varying levels of medication non-compliance on treatment outcome is indicated.



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Risk of osteoporosis and fracture incidence in female type 2 diabetic patients in Korea

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Background and aims: Several of the previous epidemiologic studies suggested that type 2 diabetes are at increased risk of osteoporotic fractures. However, there are no published data on fracture risk and related clinical features in patients with diabetes in Korea. In this study, we compared fracture incidence of type 2 diabetic female patients with that in non-diabetic hypertensive cohort.

Materials and methods: We conducted a retrospective cohort study in a secondary referral hospital. The incidence of fracture in a type 2 diabetic cohort was compared with that in a non-diabetic hypertensive cohort for 6 years. Female type 2 diabetics who visited our endocrinology department and non-diabetic female hypertensive patients who visited our cardiology department from Jan 2004 to April 2004 were assigned to the diabetic cohort and the non-diabetic hypertensive cohort, respectively. Patients with end-stage renal disease, malignancy were excluded. The data on the fracture event was obtained. Surveys on the use of anti-osteoporosis medications and bone mineral density (BMD) using DEXA scanning were done.

Results: The incidences of fracture were 111 in the female diabetic cohort (n= 1362, 60.9 ± 11.5 years) and 66 in the female non-diabetic hypertensive cohort (n=1082, 61.9 ± 11.8 years). The estimated annual incidences per 1000 persons were 14.9 and 10.5, respectively. The relative risk (RR) in diabetic cohort was 1.39 (P = 0.33; 95% CI, 1.03–1.90). The increased fracture risk in diabetes remained still significant after adjusting for age (RR 1.57; 95% CI, 1.15–2.14). There existed a difference in the fracture site between the two group; in diabetic cohort, vertebral/hip/distal radial/any other site fracture were 35.3%, 19.8%, 12.9, 31.9%, respectively; in hypertensive cohort, 51.5%, 22.7%, 18.2%, 7.6%, respectively (P=0.002). According to BMD criteria, the prevalence of osteoporosis and osteopenia in diabetic cohort (21.6% and 50.5%, respectively) was comparable that of hypertensive cohort (24.2% and 54.1%, respectively). Use of any osteoporosis medication was more common in diabetic cohort (12.1%) than in hypertensive cohort (4.3%) (P<0.001), while the proportion of bisphosphonate, sex hormone, and raloxifen use was similar between the two groups.

Conclusions: In our study, significantly higher incidence of fracture was observed in female type 2 diabetics as compared to non-diabetic female hypertensive patients despite they had similar BMD. Special concern for this fracture risk group is warranted for diabetes professionals.

PS 005 Cardiovascular risk

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Impact of introducing HbA_{1c} into the diagnostic criteria and cardiovascular risk profiles of individuals with newly diagnosed diabetes

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Background and aims: Evidence has indicated that the glycation and oxidation of lipids may contribute to the development of atherosclerosis in diabetic individuals. Although glycated hemoglobin A1c (HbA1c) was newly introduced into the new criteria for diagnosing diabetes, its performance has not been evaluated in detail, especially among diverse ethnic groups. We aimed to evaluate the impact of introducing HbA1c for diagnosis of diabetes and to investigate whether individuals with diabetes diagnosed by HbA1c criterion have different cardiovascular risk profiles than those diagnosed by fasting plasma glucose (FPG) criterion in a large cohort of Japanese individuals.

Material and methods: This cross-sectional study involved 26884 participants without known diabetes aged 20–91 years who underwent a routine medical checkup at the Health Management Center, Toranomon Hospital. Subjects were categorized into 4 groups according to the presence or absence of FPG ≥ 7.0 mmol/L and/or HbA1c $\geq 6.5\%$.

Results: Prevalence of undiagnosed diabetes by FPG and/or HbA1c was 3.6% among participants. When only HbA1c or FPG was used for diagnosis, almost equal numbers of diabetic individuals were missed (26.5% or 26.0% respectively). HbA1c $\geq 6.5\%$ criterion yielded high specificity (99%) and low sensitivity (64%) for identifying diabetes diagnosed by FPG criterion. The distribution of clinical metabolic markers such as adiposity, blood pressure, and lipids did not increase in parallel within each diagnostic group. Compared with individuals with diabetes according to FPG ≥ 7.0 mmol/L and HbA1c $< 6.5\%$, individuals with diabetes according to HbA1c $\geq 6.5\%$ and FPG < 7.0 mmol/L were characterized as older, more likely to be women, and having lower systolic blood pressure, diastolic blood pressure, and gamma-glutamyltransferase values. Individuals with discordantly diagnosed diabetes by HbA1c had unfavorable lipids profiles, such as low HDL cholesterol levels and high LDL or non-HDL cholesterol levels. A similar association was observed that we investigated characteristics of pre-diabetes defined by HbA1c (5.7–6.4%) and/or FPG criteria (5.6–6.9 mmol/L) among non-diabetic individuals. HbA1c $\geq 5.7\%$ and FPG < 5.6 mmol/L was associated with low levels of HDL-C and high levels of LDL-C and non-HDL-C. Unfavorable lipid abnormalities did not depend on the specific cutoff value of HbA1c $\geq 6.5\%$.

Conclusions: After introducing HbA1c into the diagnosis, large numbers of previously undiagnosed cases of diabetes in Japanese men and women were detected. These newly diagnosed patients had unfavorable lipid profiles, reflecting an atherosclerotic trait. Understanding the difference in risk profiles of diabetic patients by clinicians will be of great importance.

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A new cardiovascular score including diabetes for the low risk and aged southern European population: the erice risk score

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Background and aims: The impact of diabetes mellitus (DM) on cardiovascular disease (CVD) risk is an important aspect to be considered in risk assessment. The European SCORE did not consider the overall impact of DM on CVD risk. Also, it appears that true effect of DM in Europe is greater than in risk estimation systems based on the Framingham cohort where DM is included. To develop a new cardiovascular disease risk prediction tool, including the information on DM status, to accurately estimate the individual cardiovascular risk in Southern Europe, where people have a relatively low CV risk, but a relatively high prevalence of DM.

Materials and methods: The project assembled a pool of 7 Spanish cohort studies including middle-aged (30–74 years) and elderly individuals (> 75 years). There were 11,800 persons free of CVD at baseline (5,413 men and 6,387 women) representing 108,569 person years of follow-up. DM was de-

fined as FPG ≥ 7.0 mmol/L, random capillary FG ≥ 11.1 mmol/L or treatment with any anti-diabetic drug at baseline. Cox regression analyses were conducted to examine the contributions of the different variables to CVD forming the potential basis for the development of the CVD risk-score (ERICA-score). **Results:** Overall prevalence of DM at baseline was 8.6% (8.8 in males, and 8.4% in females). A total of 1,214 cardiovascular events were identified, of which 633 were fatal. Age was the strongest risk factor for CVD. With regard to modifiable risk factors, in men, high SBP was the strongest predictive factor of CVD followed by DM and smoking with similar impact. In women, DM plays a crucial role followed by smoking and high SBP. The contribution of high Cholesterol levels to the CVD risk was small, both in men and women, in this Southern population, when considering all age-groups. The multivariate adjusted hazard ratios of CVD in people with DM were 1.37 in men and 1.59 in women, compared to non-diabetics. The individual contribution of DM to the global CVD risk was higher in this Southern European population than in the Framingham population.

Conclusion: Separate risk charts are given for diabetics and non-diabetics, because at every level of a risk factor or at any combination of risk factors the absolute risk is increased in diabetic patients. The individual contribution of DM to the CVD risk is important in the Southern European population. However the Euro Heart SCORE risk function does not include DM as a risk factor score and the Framingham algorithm underestimate its impact.

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Predicting changes in risk factors in type 2 diabetes in the post-UKPDS era: longitudinal analysis of the Swedish national diabetes register

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Background and aim: The results based on the seminal UKPDS study have been the standard for health economic models for the past decade. Our aim was to provide an update accounting for changes in treatment standards and population characteristics for the application in health economic models by predicting changes of five major risk factors (HbA_{1c}, systolic BP, BMI, total cholesterol: HDL cholesterol ratio and LDL cholesterol) in persons newly diagnosed with type 2 diabetes.

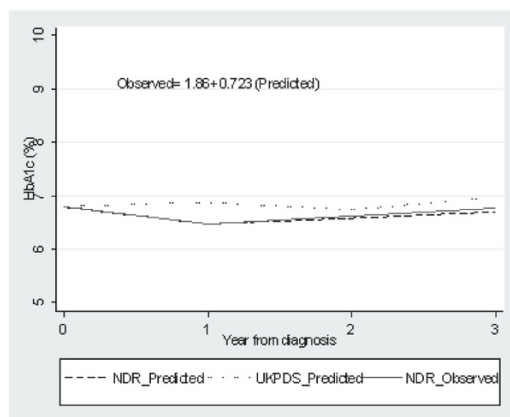
Materials and methods: Observational data from the Swedish National Diabetes Register (NDR) were analyzed using Generalized Methods of Moments estimation for dynamic panel models (N=5020, aged 25–70 years at diagnosis in years 2001–2004). Temporal validation was performed using persons diagnosed in 2005 (n = 414). Results were compared to corresponding predictions based on time path equations estimated for the UKPDS outcome model.

Results: The value of the risk factor in the previous year was the main predictor of the current value of the risk factor in all equations. These coefficients were below 1 (range 0.35–0.81) and it implied that initial high risk-levels were reduced over time while low risk-levels would increase. The rate of convergence was significantly lower for BMI than for other risk factors. BMI was associated with elevations in all other risk factors. Females had lower levels of HbA_{1c}, total: HDL cholesterol ratio and systolic BP than males. The results from the model validation showed that the predicted values corresponded well with the observed ones (Figure 1). Our updated equations predicted the current data more precisely than previous equations based on UKPDS outcome model (lower root mean squared error for NDR vs. UKPDS: HbA1c 12.6 vs. 15.2; total: HDL cholesterol ratio 13.7 vs. 16.1; systolic BP 19.0 vs. 20.2).

Conclusion: Our findings indicate new time-patterns for risk factors in the post-UKPDS era and the validation analysis confirmed the importance of updating the equations as new data become available. We suggest that the results from UKPDS trial may have affected the management of type 2 diabetes and ignoring this effect may seriously bias the results of health economic analyses of current therapies.

Figure 1. Predicted and observed time path of HbA_{1c} for non-smoker males in validation sample.

Equation shows the regression of observed values on predicted ones for this group



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Is type 2 diabetes equivalent to a cardiovascular event?

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Background and aims: There is on-going debate whether type 2 diabetes should be considered as a cardiovascular risk factor equivalent to a cardiovascular event itself. The aims of this study were to compare the mortality and comorbidity between type 2 diabetic patients with no previous cardiovascular events and patients with previous cardiovascular events considering therapy, diabetes and cardiovascular events duration, glycemic and cardiovascular risk factors control.

Materials and methods: A prospective cohort study with 3-year follow-up was performed in 2,588 patients: 1,430 with a previous cardiovascular event (from which 24% had type 2 diabetes) and 1,158 with type 2 diabetes recruited from a primary health care center in Southern Europe. Cardiovascular event was defined as a previous episode of CHD, stroke or peripheral arterial disease registered in the clinical database. The study was approved by the local ethics committee and obeyed all the laws according to the Declaration of Helsinki. As of recruitment, information was collected on cardiovascular risk factors, diabetes control, therapy, deaths and comorbidity (which was assessed through the Charlson index). Results are expressed as means \pm SD, if continuous variables, or as %, if categorical variables. X2 tests were performed for the comparison between categorical variables and ANOVA in case of continuous variables using STATA.11 software. The significance level was set up at $p < 0.05$ (two-sided).

Results: At baseline, results showed that all patients had a similar duration of diabetes and cardiovascular event since diagnosis registered in the database. At three years of follow-up, there are 855 patients with a previous cardiovascular event and without type 2 diabetes (CVnoDM), 301 with a previous cardiovascular event and type 2 diabetes (CVDM) and 1,012 with type 2 diabetes (DM). Blood pressure, LDL and total cholesterol serum levels, physical activity performed and HbA_{1c} levels in diabetics are similar in the three groups. All patients are overweight (BMI > 25), 63% are men, aged 73 years in average and an 11% are smokers. DM receive in a minor proportion flu vaccine compared to CVnoDM and to CVDM (12% vs 63% vs 74% respectively, $p < 0.001$). DM have more comorbidity associated compared to CVnoDM (Charlson's index of 2.09 (95%CI 2.00-2.18) in DM and of 1.83 (95%CI 1.72-1.93) in CVnoDM, $p = 0.02$). CVnoDM and CVDM are in a higher proportion on anticoagulation or antiplatelet therapy (67% and 77%, respectively) compared to DM (47%, $p < 0.01$). Treatment with ACEIs or ARAII is intensified in CVDM patients (64% of use) compared to CVnoDM patients (43% of use, $p = 0.003$). No significant differences in the proportions of deaths during the last year in DM (1.1%) and CVDM (3.2%) were found compared to CVnoDM (2.2%) and neither comparing DM with CVDM ($p = 0.3$). There is almost the double of cardiovascular death (27.3% vs 14.4%, $p = 0.02$) in CVDM compared to CVnoDM. There is a similar proportion of cardiovascular death comparing CVnoDM with DM (14.4% vs

16.7%, $p = 0.7$). A similar ratio of cancer death has been found in all groups (18% on average).

Conclusion: Type 2 diabetes generates more morbidity itself than cardiovascular disease without diabetes. Patients with a previous cardiovascular event have double the risk of cardiovascular death if they suffer additionally from type 2 diabetes compared to those who do not.

Supported by: MICINN

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The hypertriglyceridaemic-waist phenotype in relation to carotid artery atherosclerosis in Chinese patients with type 2 diabetes

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Background and aims: High waist circumference plus hypertriglyceridemia has been proposed as a useful tool to identify patients with increased cardiovascular risk in general populations. We examined the relationship between the hypertriglyceridemic-waist phenotype and the risk of carotid artery atherosclerosis (CAA) in Chinese patients with type 2 diabetes.

Materials and methods: 2625 consecutive diabetic in-patients (mean age 56 ± 11 years, 55% women) were included. CAA was determined by Color Doppler Ultrasonography and defined by the formation of detectable carotid artery plaques. Logistic regression analysis was applied to relate hypertriglyceridemic-waist phenotype to CAA.

Results: The prevalence of elevated waist circumference (≥ 90 cm in men or ≥ 80 cm in women) plus elevated triglycerides (≥ 1.7 mmol/L) was 26.7% and 45.2% respectively in patients with and without CAA. Compared with participants who had a waist circumference and triglycerides level below the threshold, those with the hypertriglyceridemic-waist phenotype had higher levels of systolic blood pressure (140.1 mmHg vs. 133.7 mmHg, $p < 0.05$), HbA_{1c} (8.7% vs. 7.7%, $p < 0.01$), apolipoprotein B (1.09 g/L vs. 0.97 g/L, $p < 0.05$), C-reactive protein (8.2 mg/L vs. 4.9 mg/L, $p < 0.01$), TNF- α (26.5 pg/ml vs. 16.8 pg/ml, $p < 0.05$) and mean intima-media thickness (0.08 cm vs. 0.07 cm, $p < 0.05$), but lower levels of apolipoprotein A-I (1.20 g/L vs. 1.33 g/L, $p < 0.05$). The hypertriglyceridemic-waist phenotype was associated with CAA (OR 1.30, 95% CI 1.07-1.56), after adjusting for age, sex, duration of diabetes, body mass index, smoking, HbA_{1c}, apolipoprotein B and C-reactive protein.

Conclusion: Among diabetic patients, simultaneous measurement of waist circumference and triglycerides could be used as inexpensive approach to identify individuals with increased cardiovascular risk.

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Serum peroxiredoxin 4 and cardiometabolic outcomes in the general population

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Background and aims: Previous studies suggest a role of oxidative stress in the development of metabolic and cardiovascular diseases (CVD). Recently, circulating levels of peroxiredoxin 4 (Prx4), a hydrogen peroxide degrading peroxidase, have been proposed as a novel biomarker of oxidative stress. Levels of Prx4 and its potential association with cardiometabolic conditions in the general population are unknown.

Material and methods: We included 7295 participants (aged 28-75 years; women 52.6%) from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study. Prx4 was measured by a novel immunoluminometric assay at baseline. Metabolic syndrome (MS) was defined according to the Adult Treatment Panel III criteria. Type 2 diabetes (T2D) was ascertained if one or more of the following criteria were met: (1) fasting plasma glucose ≥ 7.0 mmol/L, (2) non-fasting sample plasma glucose ≥ 11.1 mmol/L, (3) self-report diagnosis by a physician, (4) use of antidiabetic agents.

Results: Prx4 levels were significantly higher in individuals with prevalent MS (median [IQR], 0.85[0.55-1.38] vs. 0.65[0.41-1.05] U/L; $p < 0.001$), prevalent CVD (1.01[0.57-1.63] vs. 0.67[0.43-1.08] U/L; $p < 0.001$) and prevalent T2D (1.01[0.63-1.49] vs. 0.68[0.43-1.09] U/L; $p < 0.001$). During 10-years of

subsequent follow-up, we observed 407 (5.6%) incident cases of T2D, 642 (8.8%) CVD events and 460 (6.3%) deaths. In a model adjusted for age, lipids, BMI, waist circumference, systolic blood pressure, and parental diabetes, OR (95% CI) for T2D per doubling of Prx4 levels was 1.18 (1.01–1.37; $p=0.03$) in men versus 0.87 in women (0.71–1.06, $p=0.16$). In a model adjusted for Framingham risk factors, HR (95% CI) was 1.16 (1.07–1.27; $p<0.001$) for cardiovascular events and 1.21 (1.10–1.34; $p<0.001$) for all-cause mortality in the overall population. By adding Prx4 to above models, the integrated discrimination improvement, as a measure of reclassification, was 0.002 ($p=0.06$) for incident T2D in men, 0.003 ($p=0.003$) for CVD events and 0.004 ($p=0.02$) for all-cause mortality in the overall population.

Conclusion: Elevated Prx4 levels are associated with prevalent MS, CVD, and T2D. Prx4 improved prediction of future risk of cardiovascular events and all-cause mortality above Framingham risk score.

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Modifiable predictors of cardiovascular disease and mortality in Chinese with impaired glucose tolerance: 23-year follow-up of the Daqing diabetes prevention study

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Background and aims: Diabetes and impaired glucose tolerance (IGT) increase the risk of cardiovascular disease (CVD) and mortality. Thus, prevention of diabetes and the related excess CVD incidence and mortality is critical, and data about modifiable predictors of CVD and mortality in Chinese adults with IGT are needed. We examined the development and predictors of CVD and mortality among Chinese with IGT.

Materials and methods: 576 adults with IGT participated in the Daqing Diabetes Prevention Study - a clinic-randomized trial testing the effectiveness of a 6-year lifestyle intervention (1986–1992) to prevent type 2 diabetes. CVD events, defined as the first event including myocardial infarction, heart failure, sudden death, stroke or amputation, and all-cause mortality were ascertained in 542 (94%) participants over a 23-year period until December 31st, 2009. Proportional hazard analyses were used to determine predictors of each outcome.

Results: Among 488 participants who had complete data on the variables needed for this analysis, there were 186 first CVD events, 65 CVD deaths, and 141 all-cause deaths. Age, sex, smoking and changes in 2-hour plasma glucose (2hPG) from baseline to the end of 6-year lifestyle intervention (C2hPG) were significantly associated with all-cause mortality after controlling for baseline body mass index (BMI), systolic blood pressure (SBP) and 2hPG. After adjusting for baseline BMI, smoking, 2hPG, age and sex, only baseline SBP and C2hPG were significantly positively associated with CVD mortality and first CVD event. The relative risk (RR) for baseline SBP (per 10mmHg increment) was 1.13 (95%CI 1.04–1.24) for CVD mortality, and 1.07 (95%CI 1.01–1.13) for first CVD event, while the RR for C2hPG (per 1 mmol/l increment) was 2.74 (95% CI 1.00–7.49) for CVD mortality, and 2.88 (95% CI 1.67–4.99) for first CVD event. In a subset of the population with baseline lipid determinations, total cholesterol was not associated with all-cause and CVD mortality or first CVD event.

Conclusion: Changes in 2hPG at the end of the 6-year lifestyle intervention significantly predicted first CVD events, and CVD and all-cause mortality, and along with baseline SBP significantly predicted first CVD events and CVD mortality. Lifestyle interventions in IGT can prevent diabetes and better prevention outcomes for CVD events and mortality may be achievable by combining lifestyle intervention with optimal blood pressure control.

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Persistent lipid abnormalities in statin-treated patients: Portuguese diabetic sub-population of the Dyslipidemia International Study (DYSIS) P.M. da Silva¹, S.M. Cardoso²

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Background and aims: Diabetes mellitus (DM) is a well-established risk factor for cardiovascular disease (CVD). DM patients often experience multiple lipid abnormalities. This study aimed to evaluate the prevalence and type of persistent lipid abnormalities in Portuguese diabetic sub-population treated with lipid modifying therapies (predominantly statins).

Materials and methods: The Dyslipidemia International Study (DYSIS) was a multicenter, epidemiologic cross-sectional study, conducted in 12 European countries and Canada. Entry criteria included patients ≥ 45 years old, with a lipid profile assessment performed in the previous 6–12 months while on statin therapy for at least 3 months. In Portugal, along with the other countries, data concerning demographic characterization, cardiovascular risk factors and lipid modifying treatment were collected between April 2008 and February 2009 in primary care centres and private practice.

Results: In Portugal, 916 patients were recruited of which 348 (38.0%) had DM. Patients with DM had significantly lower LDL-c levels than patients without DM, however, there were no differences between the two populations regarding triglyceride and HDL-c values (Table 1). The percentage of diabetic patients that did not meet the ESC guidelines for total cholesterol and LDL goal levels was lower than the observed in patients without DM (61.8% vs 72.5%; $p<0.001$; 57.9% vs 66.3%, $p<0.05$, respectively). On the other hand, there were more DM patients with low HDL-c according to ESC guidelines (27.7% vs 18.8%, $p<0.01$). There were no differences in hypercholesterolemia treatment between patients with and without DM: the most frequently used statin was simvastatin in both groups (54.9% vs 56.5%, $p=0.64$).

Conclusion: The Portuguese results of DYSIS show that total cholesterol, triglyceride and HDL-c remain outside the recommended levels in more than 50% of statin-treated patients with DM. Although this is a high risk population for CVD events, lipid management strategies did not differ from the general population. This is in line with the international findings of this study. These patients remain at increased CVD risk and supplementary treatment may be indicated.

Table 1. Biochemical and historical results for DYSIS in Portugal.

	Patients with DM N=348 (38.0%)	Patients without DM N=568 (62.0%)
LDL-c (mmol/L)	2.7 (2.1–3.3)	3.2 (2.6–3.9)*
[Median and Quartiles]		
LDL-c <2.59mmol/L	42.1% (118/280)	24.5% (105/429)*
Triglycerides <1.69mmol/L	57.4% (193/336)	58.7% (315/537)**
HDL-c >1.03 (men)/1.29(women), mmol/L	65.8% (223/339)	71.8% (375/522)**
Systolic B.P. (mmHg)	137.4 (+/- 16.8)	136.3 (+/- 16.9) **
[Mean and S.D.]		
ACE Inhibitor treatment	41.4%	26.5%*
HbA1c (available in 304/348 - 87.4%)	7.0 (6.2–7.7)	-
Anti-diabetic therapy	67.5% (235/348)	-
Parental history DM	47.4% (165/348)	18.3% (104/567)*

* $p<0.0001$; ** $p=n.s.$;

EASD/ESC Guidelines 2007: LDL-c target = 1.8 - 2.0mmol/L if DM and CVD; Increased risk of CVD if triglycerides >1.7mmol/L, HDL<1 (men)/<1.2 (women)

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The clustering of components of metabolic syndrome, duration of onset of diabetes and coronary artery disease in type 2 diabetes mellitus based on the diabetes case management program 2001, Taiwan

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Background and aims: In order to evaluate the relationship between clustering of components of metabolic syndrome (MetS) and coronary artery disease (CAD) in different duration of T2DM for the ensuing development of CAD prevention program in high risk people.

Materials and methods: From Jan. 2006 to Jun. 2009, 3543 cases of T2DM, age over 20 were cumulatively enrolled in DCMP 2001. All patients were having the general anthropometric data collected, fasting blood samples taken and then metabolic variables measured. CAD in participants was evidenced by history of CAD and myocardial infarction and regular follow-up in CV clinics and other evidences resulting from coronary angiogram and/or echocardiogram. Duration of diabetes was calculated by subtracting time of onset of diabetes from the current age. The MetS defined was based on the criteria mentioned in the ATP III. Patients were obligatorily classified under 6 groups, MetS with waist component, waist required and non-waist required; MetS without waist component, with 2 other components and with more than 2 components; Non-MetS with waist and without waist component. All patients with CAD in 6 groups with different ranges of duration of diabetes were presented by case number and percentage distribution. By using the logistic regression analysis with Non-MetS without waist in newly developed T2DM as a reference group, the odds ratio (OR) for occurrence of CAD among 6 groups was analyzed.

Results: The case number and percentage distributions of CAD occurring in these 6 groups with different duration of diabetes were demonstrated in Table 1. The ORs for occurrence of CAD were statistically significant all the way from newly onset of diabetes to the duration of diabetes over 15 years in both non-waist required MetS with waist component ($p < 0.038-0.001$) and MetS without waist with over 3 components ($p < 0.004-0.000$) in this T2DM cohort (Table 2). The ORs for the occurrence of CAD of other 4 groups were also presented.

Conclusion: The clustering of different components of MetS rather than waist in subjects with MetS would be higher in the prevalence rate of CAD in this T2DM cohort.

Table 2. The distribution of odds ratio for the occurrence of coronary artery disease in metabolic and non-metabolic syndrome with different ranges of duration of disease in type 2 diabetes mellitus

Group	Duration (year)					
	0 (Newly)	0-5	5-10	10-15	>15	
Non-MetS, WA(-), C(>0)	OR	1	3.2	5.37	4.69	15.02
	p-value	(Reference)	0.073	0.013	0.0453	<.0001
Non-MetS, WA(+), C(>0)	OR	2.56	6.5	<0.001	11.27	8.05
	p-value	0.42	0.024	0.979	0.043	0.079
MetS, WA(-), C(>3)	OR	8.67	5.69	10.06	16.69	10.73
	p-value	0.004	0.019	0.002	0	0.012
MetS, WA(-), C(3)	OR	0.7	1.54	12.52	9.88	23.72
	p-value	0.758	0.597	<.0001	0.002	<.0001
MetS, WA(+), C(>2)	OR	4.04	11.09	13.32	18.78	20.32
	p-value	0.038	<.0001	<.0001	<.0001	<.0001
MetS, WA(+), C(2)	OR	2.47	8.21	11.46	16.1	4.17
	p-value	0.272	0.001	0.001	<.0001	0.223

WA: waist, C: components of metabolic syndrome

OR: odds ratio

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Modelling integrated care for diabetes based on observational data: the MICADO model

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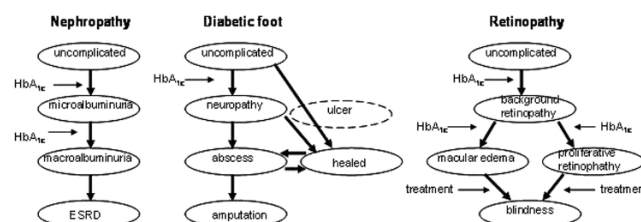
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Background and aims: Simulation models can assist in comparing the cost-effectiveness of various interventions. Already known models concentrate on known diabetes patients. However, the MICADO model was developed for the evaluation of long-term cost-effectiveness of interventions in both diabetes patients and the general population. We describe the modelling of the incidence and progress of microvascular complications in MICADO. This model combines the modelling of macro- and microvascular complications for the general population and for diabetes patients, which makes the model rather unique.

Materials and methods: The already existing and tested RIVM Chronic Disease Model was modified and extended with modules for microvascular complications (nephropathy, diabetic foot and retinopathy) in persons with type 1 or type 2 diabetes (Figure). The resulting MICADO model is a Markov-type, multistate transition model linking risk factors to incidence of diabetes and micro- and macrovascular complications. Outcomes are prevalence of complications, quality of life, costs and cost-effectiveness. Validation was performed by comparing the prevalence or incidence of microvascular complications estimated by the model to empirical data of these complications. To account for uncertainty in the transition probabilities, we performed probabilistic sensitivity analysis.

Results: The incidence of End Stage Renal Disease (ESRD) (247 (95% Interquartile Range (IR): 120 to 363)) was similar to the registered incidence of ESRD in the Netherlands in 2005 (268). The model estimated 592 (95% (IR): 291-842) amputees annually, which compared well to the registered number of diabetes related amputees in the Netherlands in 2003 (728). The prevalence of diabetes related blindness was probably slightly underestimated by our model (720 (95% IR: 690 to 748)).

Conclusions: Validation to independent empirical data showed that the model in its current form performed well. The MICADO model can be used for the simulation of a population to describe the natural course of diabetes and its complications and to evaluate the long-term (cost-) effectiveness of diabetes-related interventions aiming to reduce the risk of vascular diseases.



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Circulating levels of palmitoleic acid correlate with beta cell function in obese children and adolescents

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Background and aims: The prevalence of obesity is increasing exponentially on a global scale, not only afflicting adults but children and adolescents as well. Pathogenesis of type 2 diabetes mellitus (T2DM) is associated with obesity and is a consequence of inadequate beta-cell function and peripheral insulin resistance. In obese individuals levels of free fatty acids are elevated. Such elevated levels, in particular of palmitic acid, have detrimental effects on beta-cells. Recent studies show that another circulating free fatty acid, palmitoleic acid, has positive effects on beta-cell function and survival. In addition, the palmitoleic acid level has been shown to correlate positively with insulin sensitivity in adults. To what extent palmitoleic acid levels are altered early in development of obesity and precipitating beta-cell dysfunction and T2DM is not known. The aim of this study was to measure circulating levels of palmitoleic acid in young obese patients and correlate the fatty acid levels with beta-cell function and insulin resistance.

Material and methods: Fasting blood samples were obtained from 24 obese children, 12 boys and 12 girls, between 3 and 16 years old belonging to the Uppsala Longitudinal Study of Childhood Obesity. All children had normal fasting glucose levels and HbA1c. Six free fatty acid levels, palmitoleic acid included, were quantified by GS/MS. In addition fasting glucose, insulin and additional metabolic parameters were obtained. In half of the patients an oral glucose tolerance test was performed. To obtain a qualitative measurement of beta-cell function, the disposition index was derived from the Insulinogenic index and the Matsuda index. Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR).

Results: Fasting insulin levels varied between 5.3 to 53 µU/ml in the study group and 17% of the patients showed normal fasting insulin levels. Palmitoleic acid constituted on average 6% of the total free fatty acid amount and correlated with the total amount of free fatty acids ($P<0.01$). The relative amount of palmitoleic acids was found to correlate negatively with basal insulin secretion ($P<0.05$) and HOMA-IR ($P<0.05$), in essence relatively high levels of palmitoleic acid was associated with lower basal insulin secretion at normal glucose levels. Total amount of free fatty acids was neither found to correlate with beta-cell function nor insulin resistance. Moreover, a significant relationship was established between the amount of palmitoleic acid and the disposition index ($P<0.05$), indicating a potentially important role of circulating palmitoleic acid levels in beta-cell function.

Conclusion: We conclude that palmitoleic acid may play a role in promoting beta-cell function and thereby serve in a protective manner against onset of T2DM in young obese individuals.

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BMI cut-offs in childhood may not be valid predictors of metabolic risk: a 9-year longitudinal study

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Background and aims: Categorical cut-offs at the 85th and 95th centiles, denoting overweight and obesity respectively, are widely applied to paediatric BMI charts. They imply that BMI tracks during childhood, and their aim is to alert the clinician to health risk. Although validated in adults, such cut-offs have not been evaluated for health risk in children. The aim of this study was to establish 1. How closely metabolic status tracks through contemporary childhood and 2. How well early BMI predicts later metabolic outcome

Materials and methods: BMI (SDS and $\geq 85^{\text{th}}$ centile, both derived from 1990 UK growth standards) and a composite metabolic risk z-score, combining insulin resistance (HOMA-IR), fasting triglycerides, total/HDL cholesterol ratio and mean arterial BP, were measured annually from 5-14y in 239 children (137 boys) from the EarlyBird cohort. Consistency (tracking) of BMI SDS and metabolic risk z-score were evaluated separately from 5y to 14y

by Pearson correlation (r), and across all annual time-points (by intra-class correlation coefficient (ICC). The ability of BMI at 5y to predict future metabolic status was established using linear regression.

Results: BMI SDS tracked moderately over the nine years of observation (all years: ICC=0.84, 5y v 14y: $r=0.62$, $p<0.001$), but the metabolic risk z-score tracked relatively poorly (all years: ICC=0.54, 5y v 14y: $r=0.30$, $p<0.001$). Although metabolic risk at 14y was slightly higher in children deemed overweight/obese at 5y than in those deemed normal weight at that age (0.25 v -0.07 z-scores, $p<0.01$), BMI at 5y only weakly predicted metabolic risk at 14y ($\beta=0.10$, $r=0.14$, $p=0.03$).

Conclusion: Truly longitudinal analyses of this kind have not been conducted before in contemporary children. Categorical cut-offs drawn on BMI centile charts, denoting overweight and obesity, give the impression of consistency and predictive value that may not exist in practice. This prospective cohort study suggests that metabolic status tracks poorly and that an early measure of BMI will not reliably predict later metabolic outcome. Use of such cut-offs, particularly in early childhood, may stigmatise unnecessarily and trigger inappropriate management.

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The prevalence of the metabolic syndrome is comparable using the nation specific IDF, WHO and NCEP-ATP III criteria in Turkey

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Background and aims: The metabolic syndrome (MS) is a term for a cluster of risk factors that increase the risk of developing cardiovascular diseases (CVD) and type 2 diabetes mellitus. However, the prevalence of MS in Turkey remains largely unknown. The aim of this study is to estimate the prevalence of MS using the nation-specific IDF criteria and compare it with the modified WHO and NCEP-ATP III criteria in a large Turkish survey.

Materials and methods: Data derived from a recently completed population-based survey of 26,499 adults (age ≥ 20 years, women 63%, response rate 89%) who participated in the cross-sectional 'Turkish Diabetes, Obesity, Hypertension Epidemiology Survey-II (TURDEP-II)'. After an overnight fast, blood glucose, lipid profile, insulin, and other biochemical parameters were measured, HOMA-IR calculated, blood pressure and anthropometrics were measured, and an OGTT was performed.

Results: First of all, population-specific cut-off values of waist in male and female were calculated by plotting waist against prevalent CVD using the ROC curve. We identified 95.5 cm for male and 90.5 cm for female as the best predictive cut-points for waist (sensitivity 70%, specificity 45%, both sexes). We used these values in the IDF criteria. The WHO criteria was slightly modified by excluding microalbuminuria. Results are summarized in the table below.

Conclusion: MS is one of the major public health problems in Turkey. When population specific adjustments are made, the prevalence is comparable by different methods proposed. However, MS may differ by age, sex and living environment. Thus, one strategic approach may not fit for all.

The prevalence of MS by different criteria in the adult population of Turkey (%)			
	IDF	WHO	NCEP-ATP III
Average	29.0	25.1	22.8
Male/Female	27.7/30.6*	25.1/25.0*	17.3/26.5*
Urban/Rural	30.2/27.6*	25.5/23.6*	23.7/21.8*
Age (20-24yr/ 60-64yr)	3.9/51.9*	4.9/40.6*	2.3/45.5*
Region (North/East)	32.4/26.5*	26.4/22.8*	24.9/21.6*
Agreement	IDF vs. WHO	IDF vs. NCEP-ATP III	WHO vs. NCEP-ATP III
Overall (positive/negative)	81.7 (71.9/85.0)	89.1 (89.7/88.9)	81.9 (59.6/89.2)
Kappa	0.54*	0.72*	0.50*

* $p=0.000$

Supported by: TUBITAK

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Relation between smoking habit and presence of metabolic syndrome: effect of gender and of previous increase in body weight

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Background and aims: Since previous population studies have shown that smoking habit is related to the presence of metabolic syndrome (MS), the aim of this study was to answer the following questions: first: is there any gender difference in the relation linking smoking habit and MS? And second: given that, as known, there is an inverse relationship between body weight and smoking, does the eventual association smoking-MS remain significant after adjustment for body weight increase during the previous decades?

Materials and methods: For the purposes of this study we used a database containing the data of 1429 subjects (527M/902F) who consecutively came to the dietetic outpatient clinic of our hospital in order to obtain dietetic prescription because suffering from overweight or obesity. In all subjects MS was assessed according to the presence of MS-ATP III criteria. The records of the database also provided the annotation of body weight at the age of approximately 18 years (BMI-18) as well as of smoking habit. Former smokers from a period of less than one year were excluded and all others were included in the group of non-smokers.

Results: Prevalence of MS was significantly higher among males, compared to females (27.5% vs. 15.2%; $p=0.0001$), similar to what happened to the prevalence of smokers (28% vs. 21%, $p=0.004$). Age and increase in body weight since age of 18yr were on average similar in both sexes (45 ± 14 (SD)yr in males and 46 ± 14 yr in females; $p=ns$, and 22 ± 12 Kg in males vs. 22 ± 13 Kg in females; $p=ns$), while BMI-18 was significantly higher among males, compared to females (24 ± 3 Kg/m² vs. 22 ± 3 Kg/m²; $p=0.0001$). When comparing smokers vs. non smoking subjects, the increase in body weight since age of 18yr resulted to be lesser in the group of smoking females (19 ± 12 Kg vs. 22 ± 12 Kg; $p=0.0001$), but not among males (22 ± 13 Kg vs. 22 ± 12 Kg; $p=ns$). After adjusting for age, BMI-18, increase in body weight and family history of diabetes the relative risk for MS was significantly associated to smoking habit equally in both sexes (OR:1.77;95%CI: 1.05-2.99 among males, and OR:1.78;95%CI:1.08-2.94 among females).

Conclusion: In this population of overweight-obese patients, smoking increases the relative risk of MS by about the 80% in both genders. Such increase seems to be independent of previous body weight changes from the age of approximately 18yr.

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An altered activity in the autonomic nervous system assessed by 24-h Holter-ECG is related to both insulin resistance and visceral adiposity in healthy subjects with or without a family history of diabetes

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Background and aims: The aim of this study was to evaluate the role of the autonomic nervous system in the development of insulin resistance and type 2 diabetes (T2D).

Materials and methods: 23 healthy individuals with first-degree relatives with T2D (R) were compared with 24 control subjects without family history of diabetes (C). The groups were matched for age (46.8 ± 12.0 vs. 47.2 ± 11.7 years), BMI (25.1 ± 3.8 vs. 25.0 ± 3.1 kg/m²) and sex (M/F; 12/11 vs. 13/11). Insulin sensitivity was assessed by a hyperinsulinemic euglycaemic clamp (56 mU/m²/min). To assess the activity of the autonomic nervous system, power spectrum analysis of heart rate variability (HRV) was calculated from 24-h Holter-ECG recordings (fast Fourier transformation of R-R intervals related to normal-to-normal interbeat intervals). Computed tomography was performed to determine abdominal adipose tissue distribution.

Results: Insulin sensitivity, (M-value; mg/kg lbm/min), HbA1c, fasting blood glucose, serum insulin and adipose tissue distribution did not differ between

R and C. Total HRV spectral power (P_{tot}) and very low-frequency (P_{VLF}) power was lower in R during the 24 h ECG-recordings ($R\ 3.50\pm 0.17$, $C\ 3.61\pm 0.25$, $p=0.02$ and $R\ 3.28\pm 0.15$, $C\ 3.38\pm 0.21$, $p=0.03$, respectively). Insulin sensitivity (M-value) was negatively associated with low /high-frequency power ratio ($P_{LF/HF}$) ($r=-0.40$, $p=0.005$). The amount of visceral adipose tissue was positively associated with $P_{LF/HF}$ during the 24 h ECG-recordings ($r=0.34$, $p=0.03$). **Conclusion:** An increased ratio of sympathetic to parasympathetic nervous activity, assessed as $P_{LF/HF}$ in 24-h Holter-ECG recordings is associated with insulin resistance and visceral adiposity, and may contribute to the development of type 2-diabetes, which may be genetically linked to a reduced overall autonomic nerve activity.

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Successful implementation of a community program of screening and three year primary prevention of type 2 diabetes with lifestyle modifications: DE-PLAN study

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Objectives: To detect individuals with high risk of type-2 diabetes (T2D) in primary care setting in Spain. To implement a lifestyle modification program to prevent T2D in high risk individuals.

Methods: 1) Screening program in individuals 45-74 years old using the FINDRISC. 2) Assessment of glucose metabolism status by OGTT in those with high risk (FINDRISC >14). 3) Lifestyle intervention (diet and physical activity): 3-month intensive program plus continuous programme). Setting: health centers of Madrid, Gredos, Arévalo, Segovia, Aranda de Duero (Castilla y León), and Talavera de la Reina (Castilla la Mancha) in Spain Year evaluations: OGTT, lifestyle and cardiovascular risk changes.

Results: In the total study population, screening with the FINDRISC was completed in 3056 individuals and 956 (31.4%) of them were classified as high risk (FINDRISC>14) for developing T2D. Among the high-risk people, screened-detected T2D (SD-T2D) was observed in 99 individuals (18.9%). The prevalence of any dysglycaemic condition (IFBG or IGT or SD-T2D) in the population was estimated around .59.5 %. The 76.9% of those classified as high risk (SD-T2D excluded) were enrolled in the intervention program.

Conclusion: Prevalence of both high risk for T2D, screening-detected T2D and dysglycemic status are quite high in the Spanish population. The FINDRISC questionnaire is a useful screening tool to detect these people. After 3 follow-up years the intervention program is still ongoing. The Primary Prevention of T2D is feasible to implement at Primary Care level in Spain.

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Delay in incidence of type 2 diabetes among high-risk Spanish individuals following lifestyle intervention in real-life primary care

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Background and aims: Based on clinical trials, type 2 diabetes (T2D) can be prevented or delayed. The aim of this study was to assess the effectiveness of an active real-life primary care strategy in high-risk individuals for developing T2D.

Materials and methods: Multicenter, longitudinal cohort assessment (4 years) conducted in 18 primary health-care centres. Caucasian individuals without known diabetes aged 45-75 years were screened using (first step) the last version of the Finnish Diabetes Risk Score (FINDRISC; 0 to 26 points). A 2-hour 75 g OGTT test was carried out (second step). People with diabetes (FPG \geq 7 mM or 2hPG \geq 11.1 mM) at baseline were excluded. High-risk screenees with FINDRISC \geq 14 and/or prediabetes (IFG or IGT) based on the OGTT were invited to participate in lifestyle intervention: either (1) general information on diet and cardiovascular health without individualized programme, or (2) intensive DE-PLAN educational program (individualized or collective) periodically reinforced. OGTT was repeated yearly to determine T2D incidence.

An attempt was made to ascertain the T2D status also in people who dropped out from the programme prematurely, and they were included in the intention-to-treat (ITT) analysis. Median follow-up time was 36 months.

Results: A total of 2054 subjects were screened (81% response); 552 of them classified as having high T2D risk (26.9%), of whom 219 (39.7%) were included in the information group and 333 (60.3%) to the intensive education group. The two groups were comparable in terms of age (62/62 yrs), sex (64/68% women), BMI (31.2/31.1 kg/m²), FINDRISC score (16/16 points), FPG (5.3/5.2 mM) and 2hPG (7.1/6.9 mM). Drop-out rate was higher in the information group (11.6% vs. 9.4%, $p < 0.05$). At study close, 52 (44.4%) in the information group and 53 (25.6%) people in the intensive education group had developed T2D (42.3% relative risk reduction (RRR), $p < 0.001$). Respectively, 64 (54.7%) and 75 (36.2%) worsened their glucose status (relative decrease = 33.8%) and 6 (5.1%) and 27 (13%) shifted from prediabetes to normal glucose metabolism (relative increase = 155%). In the ITT analysis ($n = 552$ –3 deaths) the cumulative T2D incidence was 28.9 vs. 18.4% (36.3% RRR, $p = 0.004$).

Conclusion: A substantial reduction in T2D incidence can be obtained in real-life primary health care setting by intensive lifestyle intervention among high-risk subjects identified with the simple FINDRISC tool.

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Long term high dose fish oil supplementation does not alter glucose disposal in older subjects with impaired glucose regulation

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Introduction: Animal studies have shown improvements in insulin sensitivity and glucose tolerance with long chain (LC) n-3 polyunsaturated fatty acids (PUFA) in some species. Possible mechanisms for improving insulin sensitivity with fish oils include increasing the fluidity of membranes (by incorporation of LC n-3 PUFA) and by reducing inflammation. Human studies have reported a range of responses to LC n-3 PUFA. In a review of 21 studies assessing changes in insulin sensitivity with LC n-3 PUFA in humans, 2 showed a positive effect, 9 responded negatively and 10 were unchanged. Most studies with negative results reported on people with type 2 diabetes treated with oral hypoglycaemic therapy. Such variability in human studies may be due to differences in the quantity and duration of supplementation used in the experimental models and the extent of metabolic decompensation. Recent animal studies have reported that muscle membranes need to be enriched to 14% with LC n-3 PUFA for insulin sensitivity to show a significant improvement. We sought to establish the effects of 9 months of high dose LC n-3 PUFA supplementation on insulin sensitivity to glucose in a group of individuals with impaired glucose regulation (IGR).

Methods: 33 subjects (20 men, 13 women, mean age: 61 years, range: 51–68) with IGR (impaired fasting glycaemia $n = 8$, impaired glucose tolerance $n = 13$, type 2 diabetes, diagnosed at screening not requiring medication for glycaemic control and HbA1c $< 7\%$, $n = 5$ or a random glucose > 5.5 mmol/l, $n = 7$) were enrolled for a paired placebo controlled double-blind study. Mean BMI was 32.8 kg/m² (SD: 5.9 kg/m²). Subjects were paired according to sex, glucose disposal during clamp 1 and body composition. After pairing they were supplemented with 6g fish oil (3g LC n-3 PUFA) or 6g maize oil (control) daily. The dose of fish oil used in this study is the highest recommended by the UK Food Standards Agency. At the beginning and end of the study (clamps 1 and 2), subjects underwent a 2h period of glucose kinetics to assess fasting endogenous glucose production (EGP) using D-[6,6-²H₂]glucose followed immediately by 3h hyperinsulinaemic-euglycaemic clamp (40 mU/m²/min). The dextrose used in the clamp was also labelled with D-[6,6-²H₂]glucose to allow the suppressed EGP to be determined. Statistical analysis was performed using the restricted maximum likelihood approach.

Results: Mean total glucose disposal (infused plus non-suppressed EGP) did not differ between clamps 1 and 2 (6.37 vs. 6.95 mg/kg fat free mass (FFM)/min; $P = 0.18$) or the different oil supplements ($P = 0.88$) or gender ($P = 0.27$). Overall, there were no changes in fasting EGP between clamps (2.74 vs. 2.68 mg/kg FFM/min; $P = 0.33$). EGP was also not affected by supplement ($P = 0.56$) or sex ($P = 0.22$). Overall, suppressed EGP during the clamp decreased during the study (76.7% vs. 68.7%; $P < 0.01$). Men showed no differences between the two oils but in women the suppressed EGP decreased by 11.3% on fish whilst suppressed EGP did not change in the maize oil group ($P =$

0.047). The mean proportion of total glucose disposal accounted for by non-suppressed EGP was only 13.7% (SD: 9.5%).

Conclusion: Our results indicate that long term, high dose LC n-3 PUFA supplementation does not alter fasting endogenous glucose production or glucose disposal in subjects with impaired glucose regulation.

Clinical Trial Registration Number: NCT01241474

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High dietary niacin intake strongly and independently predicts decrease in liver fat during a lifestyle intervention in humans

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Background and aims: Nicotinic acid (niacin) has well known favourable effects on serum lipids in humans. This is thought to be mediated mainly through the inhibition of adipose tissue lipolysis and, thereby, the flux of fatty acids to the liver. In addition, niacin directly inhibits the activity of diacylglycerol acyltransferase 2, which catalyzes the final step in triglyceride synthesis and putatively increases hepatic lipid oxidation. Thus, it may be a promising tool to treat nonalcoholic fatty liver disease (NAFLD). Hence, we hypothesized that a high dietary intake of niacin may be associated with a larger decrease in liver fat during a lifestyle intervention.

Materials and methods: A total of 202 nonalcoholic, apparently healthy subjects at risk for type 2 diabetes and cardiovascular disease underwent a lifestyle intervention with increase in physical activity and diet modification and provided information about their diet. The composition of the diet was estimated with a validated computer program using 2 representative days of a 3-day diary. Total-, subcutaneous abdominal- and visceral adipose tissue was measured by magnetic resonance tomography and liver fat by magnetic resonance spectroscopy at baseline and after 9 months of follow-up.

Results: During the intervention total-, subcutaneous abdominal- and visceral adipose tissue and liver fat decreased (all $p < 0.0001$). The largest decrease was found for liver fat (-32%, $p < 0.0001$). However, there was a large variability in this change. A high intake of niacin strongly and independently of age, gender, body fat and energy intake predicted a larger decrease in liver fat ($p < 0.0001$) and subjects in the highest (4th) quartile of niacin intake had the largest decrease in liver fat (1st: -22%; 2nd: -21%; 3rd: -39%; 4th: -41%). This relationship was also independent of high cardiorespiratory fitness ($p = 0.0004$), another strong predictor of change in liver fat during the intervention. Furthermore, among 58 subjects with NAFLD at baseline, a resolution of NAFLD was found in 23 individuals, and for 1 standard deviation increase in niacin intake at baseline the odds ratio for resolution of NAFLD was 1.77 (95% CI, 1.00–3.43).

Conclusion: In conclusion, high niacin intake at baseline strongly predicted the decrease in liver fat during a lifestyle intervention in humans. In addition, niacin intake was associated with change in liver fat independently of adiposity, total energy intake and cardiorespiratory fitness, suggesting specific effects of niacin on hepatic lipid metabolism.

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Leisure time physical activity limits inflammation in individuals with impaired glucose tolerance

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Background and aims: Inflammatory markers have been associated with type 2 diabetes (T2D) and impaired glucose tolerance (IGT). There is evidence that lifestyle interventions, using weight loss and physical activity, prevent or postpone progression to T2D in individuals with IGT. However, it is unknown whether leisure time physical activity (LTPA) is associated with inflammation among patients with IGT in a representative population.

Methods: Questionnaires, including data concerning LTPA (4-graded scale; 1+2 defined as sedentary, 3+4 defined as physically active), anthropometric measures, an oral glucose tolerance test (OGTT), and C-reactive protein (CRP) were collected from a random sample of 2816 individuals, 30–75 years old, in a cross-sectional study from the South-west of Sweden.

Results: The prevalence of IGT was 7.6% (n=213). CRP was significantly higher in women with IGT compared to women with normal glucose tolerance; mean difference 1.5 (CI 0.7:2.3), $p<0.001$. However, this was not seen in men, $p=0.290$. Also in sedentary women with IGT levels of CRP were significantly higher; $\Delta 1.8$ (0.8:2.8), but not in those who were physically active $\Delta -0.4$ (-1.5:0.8). Correspondingly, CRP was higher in sedentary men with IGT $\Delta 1.3$ (0.03:2.6), but not in physically active men $\Delta 0.4$ (-1.1:1.8). Self-reported LTPA was inversely associated with CRP ($p=0.024$), which was supported by a consistent association between resting heart rate (proxy for fitness) and CRP ($p=0.001$). All tests were adjusted for differences in age, BMI, and smoking.

Conclusion: In an unselected population physical activity is associated with low systemic inflammation in individuals with IGT.

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Risk factors in the Portuguese population with diabetes: diabetes prevalence study in Portugal

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Background and aims: The objectives of this study were to determine the prevalence of risk factors in type 2 diabetes in the Portuguese population aged between 20 and 79 years

Materials and methods: Taking into account the number and distribution of inhabitants (7,657,529 people aged between 20 and 79 years), 122 representative units were selected. The total sample participating in the study comprised 5,167 subjects. Data were standardized for the Portuguese population aged 20–79 years. Arterial hypertension (IDF/ESC/EASD) systolic values ≥ 130 mm hg and diastolic ≥ 80 mm hg were considered hypertension. Excess weight and obesity - were defined using the BMI (body mass index), IDF/ESC/EASD criteria: Excess weight ≥ 28 kg/m² and obesity ≥ 30 kg/m². Abdominal perimeter ≥ 94 in men and 80 in women was considered abnormal (IDF criteria).

Results: Arterial hypertension - (TA $\geq 130/80$) was found in 70.9% (95% CI 68.0% to 74.0%) of diabetic people in both sexes, 75.9% in males and 66.4% in females. Only 46.8% of the men and 58.4% of the women with arterial hypertension were taking anti-hypertensive medication. LDL cholesterol - Only 10.8% (95% CI 7.8% to 13.0%) of the women with diabetes had LDL Cholesterol <100 mg/dl and only 15.5% (95% CI: 12.4% to 19.1%) of the men with diabetes had LDL Cholesterol <100 mg/dl. Triglycerides - were < 150 mg/dl in 57.7% (CI 95%: 54.4%-60.8%) of people with diabetes, with or without medication. HDL - Cholesterol - 39.7% (95% CI: 35.3% to 44.4%) of the female population had values < 50 mg/dl and 21.2% (95% CI: 17.7% to 25.2%) of the males had values < 40 mg/dl. Abdominal perimeter (AP) - The average abdominal perimeter in diabetic men was 102.4 cm (95% CI 101.3 to 103.4); median = 103. It was > 94 cm in 77.1% of the total. The average AP in non-diabetics was = 97 (95% CI 96.4 to 97.6) median 97, with AP > 94 cm in 59.6% of the total. In diabetic women, the average abdominal perimeter was 101.1 (95% CI 99.9 to 102.2); median = 101. In 96.3% of all diabetic women it was > 80 cm. The average AP of non-diabetic women was = 92.8 (95% CI 92.3 to 93.3) median 92.2. Body Mass Index (BMI) - A significant difference was detected between the figures in diabetic people and non-diabetics and also between men and women. Non-diabetic men have an average BMI of 27 (95% CI 27.3 to 27.7), diabetic men - average BMI 29.3 (95% CI 29.0 to 29.7), variance analysis - $F=65.247$; $p<0.0001$; the figures for women are: average BMI in non-diabetic women 28.2 (95% CI 28.0 to 28.4) and in diabetic women 31 (30.5 to 31.5) - variance analysis - $F=105.6253$; $p<0.0001$.

Conclusion: Diabetes is a chronic disease with a high prevalence in Portugal, one of the highest in Europe. In addition to glycemic control there is a marked failure in the control of risk factors particularly blood pressure, dyslipidemia and obesity. A significant number of people take medication but remain uncontrolled. Strategies to underline these factors must be implemented not only among patients but also among health professionals.

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Prevalence of abnormal glycaemia and its associated risk factors in rural adult Bangladeshi population

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Background and aims: Substantial racial heterogeneity in diabetes leads to the necessity of conducting epidemiological studies in different communities. Such studies are still inadequate in Bangladeshi population, particularly in

truly respective rural areas. The objective of the present study was to explore the prevalence of diabetes, prediabetes and their associated risk factors in a rural adult Bangladeshi population.

Materials and methods: This population based cross-sectional study was conducted through diabetes screening camps arranged in remote rural areas of North Western Bangladesh, which included a total of 836 participants (468 male, 368 female), aged at or above 30 years. Clinical and anthropometric data were collected by using appropriate techniques. WHO guideline for Asian population was used to identify overweight and obese. Diabetes, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were diagnosed by WHO criteria after a 2 sample OGTT. Serum glucose was estimated by glucose oxidase method. Multiple regression analysis was used with adjustment for potential confounders.

Results: The prevalence of diabetes and prediabetes (both IGT & IFG) were 7.2% (95% CI 5.4–9.0) and 6.5% (95% CI 4.8–8.2) respectively. It differs between male and female, but it increased with age. The mean BMI was significantly higher in the diabetic (23.9 ± 4.7) and prediabetic (23.1 ± 4.4) groups compared to the nondiabetic group (21.7 ± 3.6 ; $p < 0.006$ – 0.002). A similar difference was found in case of WHR (0.97 ± 0.09 in diabetic, 0.92 ± 0.06 in prediabetic and 0.89 ± 0.07 in nondiabetic groups, $p < 0.05$). On Pearson's correlation analysis FBG ($r = 0.119$, $p = 0.001$) and 2HBG ($r = 0.125$, $p = 0.001$) both showed significant correlation with BMI. FBG ($r = 0.226$, $p = 0.001$) and 2HBG ($r = 0.125$, $p = 0.001$) also showed significant correlation with WHR. On multiple regression analysis when the confounding effects of age and sex were adjusted, BMI and WHR showed significant association for the occurrence of diabetes in this population.

Conclusion: A high prevalence of both diabetes and prediabetes exists in rural population and it seems to be associated with obesity. Preventive programs, particularly targeted to body weight management through lifestyle modification should be strengthened even in remote rural areas.

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Incidence trend of type 2 diabetes in children and adolescents in Germany

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Background and aims: An increasing incidence of type 2 diabetes in children and adolescence has been observed in native populations. A rising occurrence of type 2 diabetes is also under discussion in Europe. Aim of the present study was to estimate incidence and time trend of type 2 diabetes in children 5–19 years of age in the large risk population of the German federal state North Rhine-Westphalia (NRW) during 2002–2009.

Materials and methods: During the study period the average risk population was 2.94 million children. The North Rhine-Westphalian diabetes incidence register ascertains newly diagnosed cases of type 2 diabetes by means of three data sources: the prospective hospital-based active surveillance system ESPED, annual inquiries among paediatric, internal and general medical practices, and the computer-based documentation system DPV founded for quality control and scientific research in paediatric diabetes care. Completeness of ascertainment was estimated by the capture-recapture-method. Point and interval estimates (95%-CI) of incidence rates (per 100,000 person-years) were based on Poisson distribution and corrected for ascertainment. Age- and sex-standardized rates were estimated by the direct method using equal weights. Poisson regression analysis was applied to assess time trends.

Results: During 2002–2009 a total of 233 newly diagnosed diabetic children aged 5–19 years (98 boys, 135 girls) were registered. Ascertainment was estimated to be 82.3% (95%-CI: 74.2%–92.5%) complete. The age- and sex-standardized overall incidence rate was 1.20 (1.07–1.34). The age-standardized incidence among boys (1.03, 0.85–1.21) was lower than among girls (1.38, 1.17–1.59, $p = 0.005$). Incidence depended significantly on age ($p < 0.001$). Age-specific estimates for age groups 5–9, 10–14, and 15–19 years were 0.26 (0.15–0.38), 1.24 (1.00–1.49), and 2.1 (1.79–2.41), respectively. Annual incidence rates ranged between 0.80 in 2008 and 1.74 in 2009. No significant time trend in the incidence was observed, the average annual incidence increase was estimated as 0.2% (–4.9%–5.6%, $p = 0.934$). The incidence did also not significantly change over time in boys (annual increase: 1.8% (–6.0%–10.2%, $p = 0.669$), girls –0.1% (–7.6%–6.3%, $p = 0.790$), and the three age-groups (5–9

years: –13.9% (–30.2%–6.1%, $p = 0.160$), 10–14 years: 2.9% (–5.8%–12.3%, $p = 0.530$), 15–19 years: 0.4% (–6.3%–7.6%, $p = 0.913$).

Conclusion: Based on a large risk population, no significant change in the incidence of type 2 diabetes in children and adolescents could be observed in this study. However, since the annual number of newly diagnosed type 2 diabetic patients is very small, the trend should be followed for a longer time period. Undiagnosed cases of type 2 diabetes were not covered by this study, thus the incidence level is underestimated to a certain extent. But the under-coverage can be assumed not to affect the incidence trend seriously. Based on the actual incidence estimate, about 150 children in the age-group 5–19 years are newly diagnosed with type 2 diabetes per year in Germany.

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Racial disparities in the age of diagnosis of type 2 diabetes mellitus and impaired glucose regulation in a multi-ethnic high-risk population in Singapore

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Background and aims: The prevalence of type 2 diabetes mellitus has been increasing rapidly in Asia. Asians, especially individuals of Indian (South Asian) descent, are at greater risk of developing diabetes and cardiovascular disease. However, the effect of ethnicity on age of diagnosis of diabetes in Southeast Asia has not been established. We aimed to compare the prevalence and age at diagnosis of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes in different races in a high-risk Southeast Asian population in Singapore.

Materials and methods: All patients without a history of diabetes who had a 75g oral glucose tolerance test and concurrent HbA1c performed in our tertiary hospital from January 2001 to October 2009 were included in the study. All patients were deemed to be high-risk due to positive family history of diabetes, previous gestational diabetes, random plasma glucose level > 7.0 mmol/L, obesity or polycystic ovarian syndrome. Data was obtained through review of case records and laboratory databases. Diabetes mellitus, IFG and IGT were diagnosed based on the World Health Organization criteria. Differences between races were tested with one-way analysis of variance and multiple comparisons with post-hoc Bonferroni correction in SPSS 16.0. All values are given as mean \pm SD. A p value < 0.05 was considered significant.

Results: There were 659 patients (70.7% Chinese, 19.7% Malays, 9.6% Indians) (343 males, 52.0%). Mean age was 54.2 ± 15.4 years (range 14–96) and mean body mass index (BMI) 26.2 ± 5.8 kg/m² (range 14.7–52.7). Five hundred and thirty four patients (81.0%) had impaired glucose regulation (IGR) or diabetes. Patients of Indian ethnicity were diagnosed with diabetes and IGR at a significantly younger age than Chinese and Malay patients, and had higher fasting plasma glucose (FPG) ($p = 0.02$) and HbA1c ($p = 0.04$) than the Chinese majority (Table 1).

	Chinese (n = 369)	Malay (n = 111)	Indian (n = 54)	p value (Malay vs Indian)	p value (Chinese vs. Indian)
Prevalence (%) of diabetes and IGR	77.2 (369/466)	85.3 (111/130)	85.7 (54/63)	NS	NS
Age	56.7 ± 15.5	52.4 ± 13.3	47.6 ± 13.6	0.02	< 0.001
BMI (kg/m²)	25.4 ± 4.8	28.8 ± 7.1	27.0 ± 5.5	NS	NS
FPG (mmol/L)	7.14 ± 2.49	7.29 ± 2.22	7.87 ± 2.66	NS	0.02
2HPG (mmol/L)	14.04 ± 5.10	13.52 ± 5.74	14.33 ± 5.17	NS	NS
HbA1c (%)	6.97 ± 1.55	7.14 ± 1.53	7.37 ± 2.02	NS	0.04

Table 1. Comparison of age, BMI, FPG, 2HPG and HbA1c between the races in Southeast Asian subjects diagnosed with diabetes and IGR (n = 534). 2HPG = 2-hour post-load plasma glucose. NS = not significant.

Conclusion: Indian patients with risk factors for diabetes present at a younger age with more severe dysglycaemia compared with Chinese and Malay patients, despite no significant difference in BMI, and may need earlier screening. It is important to consider the racial disparities and differing risks of developing diabetes amongst different races when screening a high-risk multi-ethnic population.

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Diabetes epidemic in Turkey: results of the second population-based survey of diabetes and risk characteristics in Turkey (TURDEP-II)

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Background and aims: Because of the rapid change in lifestyle in Turkey, there is concern of emerging diabetes epidemic. We conducted the 2nd Turkish Diabetes Epidemiology Study (TURDEP-II) to determine the prevalence of diagnosed and undiagnosed diabetes (DM), preDM and their time trends, and to identify risk factors associated with DM in adult Turkish population. The field survey was performed from Jan to July 2010, approx. 12 yrs after the 1st survey, TURDEP-I

Materials and methods: A population-based random sample of 26,499 adults (≥20 yrs, women 63%, response rate 89%) participated in this cross-sectional survey. After an overnight fast, fasting blood glucose (FBG), and other biochemical parameters were measured in all participants, then an OGTT was performed to identify undiagnosed DM and preDM (impaired fasting glucose, IFG or impaired glucose tolerance, IGT) in non-diabetic participants.

Results: The prevalence of DM was 16.5% (undiagnosed 7.5%), translating to 6.5 million adults with DM in Turkey. The prevalence of DM age-standardized to TURDEP-I, world and European populations was 13.7%, 17.1% and 15%, respectively. The prevalence was higher in women than in men ($p=0.004$). The prevalence of isolated IFG, IGT and combined preDM was 14.5%, 7.9%, and 8%, respectively, and that of obesity 34.2% and hypertension (HT) 31.3%. Compared to TURDEP-I; DM, IGT, and obesity increased by 90%, 106% and 40%, respectively. During the 12 yrs weight and waist increased on average by 8 kg and 7 cm in men, and by 6 kg and 6 cm in women. Multiple logistic regression analysis revealed that in female; age (OR; 95%CI: 1.046; 1.041–1.050), waist (1.019; 1.014–1.024), BMI (1.016; 1.005–1.028), HT (2.008; 1.635–2.465), and living in regions other than the North were independently associated with prevalent DM, while living in rural (0.883; 0.798–0.977), less number of meals (0.347; 0.270–0.445), high education level (0.454; 0.343–0.599), and quit smoking (0.700; 0.522–0.939) were associated with less risk of DM. On the other hand, in male; age (1.048; 1.042–1.054), waist (1.011; 1.004–1.019), BMI (1.048; 1.027–1.070), HT (1.797; 1.334–2.420), living in the South and the West (1.358; 1.109–1.663), sedentary lifestyle (1.379; 1.128–1.685), family history of DM (2.522; 2.196–2.897) were independently associated with prevalent diabetes; whereas living in rural (0.769; 0.670–0.882), less number of meals (0.321; 0.231–0.447), and being widowed or divorced (0.531; 0.304–0.927) were associated with less risk of DM.

Conclusion: These results from one of the largest nationally representative survey carried out thus far indicate that DM has rapidly become a major public health challenge in Turkey and underline the urgent need for the development of national strategies aimed at the prevention, detection, and treatment of DM in the general Turkish population.

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Prevalence of diabetes and impaired glucose regulation in Spain: Di@bet.es study

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Background and aims: The Di@bet.es study is a national, cross-sectional population-based survey of diabetes, obesity and other cardio metabolic risk factors and their association with lifestyle, promoted by the Spanish National Diabetes Strategy and carried out by the Spanish Society of Diabetes (SED) and CIBER of Diabetes and Related Metabolic Diseases (CIBERDEM).

Design: Population-based, cross-sectional, cluster sampling. Target population: The entire Spanish population. Sample: 5728 subjects in 100 clusters (health centers or the equivalent in each region) randomly selected with a probability proportional to population size. Participation: 57%. Variables: Clinical and demographic structured survey, lifestyle survey (physical activity and food consumption frequency questionnaires), quality of life survey, physical examination (weight, height, BMI, waist, hip, blood pressure), oral glucose tolerance test (75g) (WHO 1999 criteria).

Results: About 30% of the population had some carbohydrate disturbance. The overall prevalence of DM adjusted for age and sex was 13.8% (CI95%= 12.8–14.7%). Of these, about half had unknown diabetes: 6.0% (CI95%= 5.4 to 6.7%). The age and sex adjusted prevalences of isolated IFG, isolated IGT and combined IFG-IGT were 3.4% (CI95%= 2.9–4.0%), 9.2% (CI95%= 8.2–10.2%) and 2.2% (CI95%= 1.7–2.7%) respectively. The prevalence of diabetes and other disorders increased significantly with age ($p < 0.0001$) and was higher in men than in women ($p < 0.001$).

Conclusion: The di@bet.es study shows, for the first time, prevalence results of Diabetes and Impaired glucose regulation in a representative sample of the Spanish population.

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Comparison of measures of general adiposity and body fat distribution as predictors of type 2 diabetes in Korean adults

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Background and aims: Not only general measures of obesity, such as body mass index or percent body fat, but also measures of central body fat distribution, such as waist circumference or waist-to-height ratio, are strongly associated with the risk of type 2 diabetes. However, it is not clear which anthropometric measurements of obesity best predict risk of type 2 diabetes. We compared various anthropometric measures of obesity and body fat distribution for their ability to predict development of diabetes, and investigated whether BMI modified the effect of body fat distribution on diabetes risk in Koreans.

Materials and methods: We analyzed the anthropometric and laboratory data of 9,432 Korean adults (age 20–79 years, 6,260 men and 3,172 women) who underwent routine medical check-ups in 2005 (baseline) and in 2010 (follow up). After excluding patients with diabetes at baseline, 8,748 subjects (5,707 men and 3,041 women) were included for the analysis. Body weight, height, waist circumference, and bioelectrical impedance (to calculate fat mass and percent body fat) were measured at baseline. Diabetes was defined as fasting plasma glucose ≥ 7.0 mmol/l, HbA1c $\geq 6.5\%$, or taking anti-diabetic medica-

tions. Receiver-operating characteristic (ROC) analysis was used to compare the ability of the parameters to predict development of type 2 diabetes.

Results: Among the 8,748 participants who were non-diabetic at baseline, a total of 308 subjects (242 men and 66 women) developed diabetes during 5 years. Each of the anthropometric parameters of general obesity (body mass index [BMI], fat mass, percent body fat) and central body fat distribution (waist circumference [WC] and waist-to-height ratio [WHtR]) was a good predictor of type 2 diabetes. However, when area under the ROC curves (AUCs) were compared, BMI (0.697, 0.669–0.725 [95% CI]), WC (0.709, 0.682–0.736), and WHtR (0.718, 0.692–0.743) were better predictors of diabetes risk than fat mass (0.672, 0.643–0.700) or percent body fat (0.657, 0.628–0.686). There was no significant difference between BMI, WC, and WHtR. When the participants were divided into 3 subgroups according to BMI, WHtR and WC were better predictors than BMI or fat mass in the low BMI (< 23 kg/m²) group. In the group with BMI > 27 kg/m², there was no significant difference between WHtR, WC, and BMI.

Conclusion: Throughout its range, BMI, WC, and WHtR were excellent predictors of type 2 diabetes in Koreans. However, in subjects with low BMI, measures of central body fat distribution is more useful than measures of general obesity in predicting type 2 diabetes risk. Table. Area under the ROC curves (AUC) for each of the anthropometric parameters in predicting type 2 diabetes

	AUC (95% CI)		
	BMI < 23 kg/m ²	BMI 23–27 kg/m ²	BMI > 27 kg/m ²
WHtR	0.706 (0.630–0.782) ^{a,b}	0.610 (0.570–0.650) ^{a,b,c}	0.593 (0.532–0.653) ^{b,c}
WC	0.692 (0.619–0.764) ^a	0.595 (0.553–0.637) ^{a,b,c}	0.581 (0.525–0.638)
BMI	0.581 (0.499–0.663)	0.549 (0.508–0.590)	0.586 (0.524–0.648) ^{b,c}
Fat mass	0.614 (0.542–0.685)	0.534 (0.491–0.557)	0.527 (0.465–0.589)
% Fat	0.636 (0.558–0.715)	0.533 (0.490–0.575)	0.521 (0.459–0.583)

^aP < 0.05 vs. BMI, ^bP < 0.05 vs. fat mass, ^cP < 0.05 vs. %fat WHtR, waist-to-height ratio; WC, waist circumference; % fat, percent body fat

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Risk determinants for type 2 diabetes

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Background and aims: Diabetes is a growing worldwide problem. In 1995 the prevalence of diabetes in Portugal was estimated to be about 5.1% of the population. In 2010 the first national study on the prevalence of diabetes (PREVADIAB) revealed a prevalence of 12.3% of the adult population aged between 20 and 79 years old. With this subsequent analysis we aimed to find risk determinants for type 2 diabetes.

Materials and methods: Using the 2001 Portuguese Census, a random sample of people aged between 20 and 79 years was selected from 122 locations representative of the distribution of the Portuguese population. Demographic characteristics were registered and an OGTT was performed. Diabetes WHO criteria were used for the diagnosis of diabetes.

Results: The total sample consisted of 5167 people that corresponds to an 83.8% response rate. A total prevalence of diabetes (diagnosed and undiagnosed) of 12.3% (CI 10.8–12.6%) was found with 43% of undiagnosed cases. The highest prevalence was in male gender (14.6% vs 10.2% in females) and in older groups (2.0% in the group 20–39 years, 12.8% in the group 40–59 years old and 27.1% in the 60–79 years old). People with the lowest level of literacy (not finishing first level of education) had a prevalence of 30.3%, different from the ones with the first level (19.4%) and the secondary level (7.9%) and university level (6.6%). In the group with BMI >30 the diabetes prevalence was 19.5%, also different from the group with BMI between 25–30 (12.1%) and the group with normal BMI (5.5%).

Conclusion: The diabetes growing prevalence raises governments' and health systems' concerns. In this study we showed an average prevalence of 12.3% in an adult population. As in other countries gender differences were found, higher in men than women. According to the known physiopathology of type 2 diabetes, we also found that the prevalence of diabetes has a strong correlation with BMI. However, the greatest difference between groups was the literacy level, showing that the lowest level of literacy was related to the highest prevalence of type 2 diabetes. This could be one of the greatest risk determinants for type 2 diabetes.

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Vitamin D levels and type 2 diabetes in a Spanish population

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Background and aims: Several studies suggest a complex relationship between the presence of insulin resistance or diabetes and vitamin D / iPTH levels. The aim of this study has been to evaluate the relationship between vitamin D and iPTH levels and the status of carbohydrate metabolism in a representative sample of the Spanish population.

Materials and methods: The study was conducted in two population of northern and southern Spain, evaluated in the same period of time and with a similar methodology: The Pizarra study (Málaga) and Asturias study. Subjects in both groups underwent clinical and anthropometric assessment survey, a blood test (fasting glucose and insulin, creatinine, calcium, phosphorus, 25OH vitamin D, iPTH) HOMA-IR (homeostatic model assessment-Insulin resistance) and an OGTT (oral glucose tolerance test). The number of subjects included was 1182. To calculate the statistical difference between the means of continuous variables we used the ANOVA test for quantitative and the Chi2 test for qualitative variables. The strength of association between two variables was measured using the Odds Ratio (OR).

Results: The mean age of participants was 50.3 ± 14.4 years (range: 20–83 years), with 57% female and 43% male. The mean of 25-hydroxyvitamin D and iPTH were: 22.46 ng / mL and 42.29 pg / mL, respectively. 65.6% had normal fasting glucose and OGTT, 6.45% had impaired fasting glucose, 8.15% carbohydrate intolerance, 9.85% new diagnosed diabetes and 9.93% known diabetes. 25-hydroxyvitamin D levels in these groups were, respectively: 23.43, 21.45, 22.97, 21.82 and 23.22 (p 0.034) and iPTH : 45.1, 48.26, 45.99, 49.31 and 41.08 (p0.001)both adjusted for age, sex, BMI and season. The percentage of patients with 25- hydroxyvitamin D levels below 20 ng / ml was different in the five groups: 30.9%, 35.5%, 37.5%, 49.1% and 36.8%(p0.014). Vitamin D levels were significantly different for different values of HOMA-IR: for quartile 1 (Homa <1.07): 25.85; for quartile 2 (Homa 1.07– 1.73): 22.94; for quartile 3 (1, 74– 2.95): 22.54 and for quartile 4 (> 2.95): 22.05 (p 0.041, all adjusted for age, sex, BMI and season).

Conclusion: 1. One-third of the subjects studied have 25 hydroxyvitamin D levels below 20 ng / mL, 2. The higher the insulin resistance (measured by HOMA-IR) the lower the levels of vitamin D, 3. Patients with undiagnosed diabetes have 25hydroxyvitamin D levels significantly lower and iPTH significantly higher than normal population

PS 008 HbA_{1c} in prediction and diagnosis

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The OGTT is a better tool for detection of diabetes, impaired glucose regulation and impaired insulin secretion and action than HbA_{1c}: the GENFIEV study

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Background and aims: HbA_{1c} (A1c) has been proposed for the diagnosis of diabetes (DM) and identification of individuals at risk of DM. Very few studies evaluated whether A1c performs better than OGTT for categorization of impaired glucose regulation (IGR: IFG, IGT, IFG+IGT) and associated metabolic abnormalities. Therefore, aims of the study were to compare these indices in their ability to identify IGR and DM and evaluate the relation of these measures to other metabolic characteristics, particularly insulin resistance and β -cell function.

Materials and methods: We have examined 844 consecutive subjects (44% men; age 49.5 \pm 11 years; BMI 29 \pm 5 Kg/m²) enrolled into the Genetics Physiopathology and Evolution of Type 2 diabetes (GENFIEV) study. Plasma glucose and C-peptide were determined during OGTT to assess β -function while lipid profile; HOMA-IR and A1c (HPLC) were evaluated in fasting condition.

Results: Based on ADA criteria 43% had normal glucose tolerance (NGT), 42% IGR and 15% DM on OGTT, while IGR and DM were 38 and 11% on A1c with a concordance rate between of 54% and 44%, respectively. A1c specificity was 74% and 95% for IGR and DM. In non-diabetic subjects (NGT+IGR) both A1c and 2h-postload glucose were correlated with lipid profile, blood pressure, obesity, although the 2-h glucose correlation was stronger (triglycerides: $r=0.21$ vs. 0.11 ; HDL-Ch $r=0.19$ vs. -0.10 ; SBP $r=0.25$ vs. 0.15 ; DBP $r=0.20$ vs. 0.07 ; all $p<0.04$) with the exception of BMI: $r=0.08$ vs. 0.13 ($P=NS$). In a logistic regression analyses adjusted for age, sex, and BMI, individuals with IGR and even more DM by OGTT had greater chance to have insulin resistance and impaired insulin secretion (Odd Ratio for HOMA-IR: IGR-OGTT 3.33 (95%CI 2.32-4.78) vs. IGR-HbA_{1c} 2.52 (1.77-3.58), DM-OGTT 8.02 (4.78-13.45) vs. DM-HbA_{1c} 3.95 (2.30-6.77); OR for Insulinogenic Index: IGR-OGTT- 3.08 (2.15-4.40) vs. IGR-HbA_{1c} 2.88 (2.03-4.09), DM-OGTT 14.24 (8.49-23.89) vs. DM-HbA_{1c} 8.56 (5.00-14.64)). Finally, IGR and DM by OGTT were more associated with metabolic syndrome (ATP III) than IGR and DM by HbA_{1c} (IGR-OGTT 2.80 (1.90-4.14) vs. IGR-HbA_{1c} 2.08 (1.42-3.04); DM-OGTT 4.31 (2.55-7.31) vs. DM-HbA_{1c} 3.36 (1.92-5.88)).

Conclusion: A1c identifies a smaller proportion of individual at-risk and even smaller with DM than OGTT and has a weaker correlation with pathogenetic defects of hyperglycemia as well as of other metabolic abnormalities. Clinical Trial Registration Number: NCT00879801

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Screening using various cutoffs of HbA_{1c} and impaired fasting plasma glucose for predicting future diabetes: the Toranomon hospital health management center study

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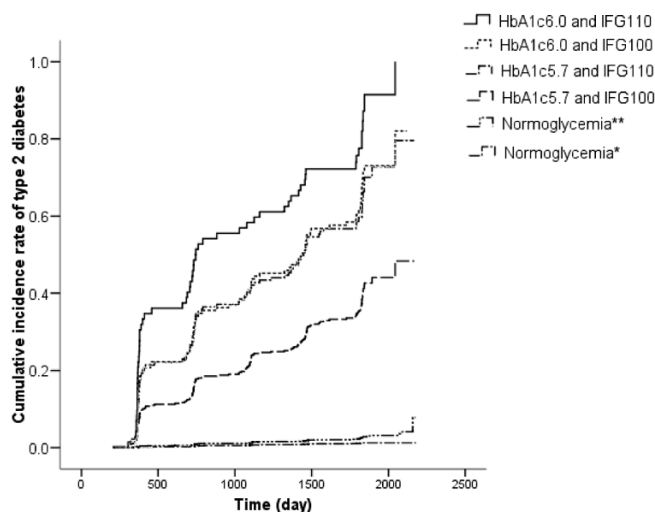
Background and aims: Complex histories in the diagnosis of non-diabetic hyperglycemia or pre-diabetes make it difficult to target persons most likely to progress to diabetes, and thereby to efficiently enable early intervention. We aimed to evaluate the performance of different screening criteria for pre-diabetes as predictors for diabetes to identify which combination of impaired fasting glucose (IFG) and elevated HbA_{1c} criteria is the most predictive of future type 2 diabetes in a large cohort of Japanese individuals.

Material and methods: The study enrolled 4670 men and 1571 women aged 24-82 years without diabetes (diabetes: fasting plasma glucose (FPG)

≥ 126 mg/dl, HbA_{1c} $\geq 6.5\%$, or self-reported clinician-diagnosis diabetes). Diagnosis of pre-diabetes was made according to IFG (FPG 100-125 mg/dl, IFG100; or FPG 110-125 mg/dl, IFG110) or elevated HbA_{1c} (5.7-6.4%, HbA_{1c}5.7; or 6.0-6.4%, HbA_{1c}6.0). A combination of two tests with alternative cut-offs was used for diagnosing pre-diabetes. The follow-up duration was a median of 5.0 years on an annual basis, during which 338 incident cases of diabetes occurred.

Results: The prevalence of pre-diabetes was 33.5% by a combination of HbA_{1c}5.7/IFG100 criteria, 28.0% by HbA_{1c}6.0/IFG100, 16.7% by HbA_{1c}5.7/IFG110, and 8.2% by HbA_{1c}6.0/IFG110. Screening by the IFG100/HbA_{1c}5.7 criteria yielded the highest sensitivity of 86%, but the lowest specificity of 70% for predicting future diabetes. Among the individuals classified as having pre-diabetes by any of the four combined criteria, 28.1-32.0% reverted to normoglycemia at the last follow-up examination. At 170.3 months (5.6 years) after a baseline examination, however, pre-diabetic individuals who fulfilled both (1) HbA_{1c}5.7 and IFG100 showed 50% cumulative risk of diabetes, both (2) HbA_{1c}5.7 and IFG110 or (3) HbA_{1c}6.0 and IFG100 showed 80%; and both (4) HbA_{1c}6.0 and IFG110 showed 100% cumulative risk (see Figure 1). Pre-diabetic individuals who fulfilled the (1), (2), (3) and (4) criteria showed 50% of cumulative incidence rate at 5.6 years, 3.9 years, 3.9 years and 2.0 years after the baseline examination, respectively.

Conclusions: Screening for pre-diabetes using a combination of HbA_{1c} 5.7-6.4% and FPG 100-125 mg/dl criteria substantially reduced the likelihood of missing future cases of diabetes. Targeted screening of pre-diabetic individuals who strictly fulfill both HbA_{1c} 6.0-6.4% and FPG 110-125 mg/dl criteria would predict definite progression to diabetes. Figure: Cumulative incidence of diabetes during follow-up time according to the baseline diagnosis of pre-diabetes using elevated HbA_{1c} and IFG (Normoglycemia*: HbA_{1c} <6.0% and FPG <110 mg/dl, Normoglycemia*: HbA_{1c} <5.7% and FPG <100 mg/dl)



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Does the HbA_{1c} cut-off point of 6.5% fit for Asia? A meta-analysis and system review

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Background and aims: Hemoglobin A1c (HbA_{1c}) $\geq 6.5\%$ was confirmed to be a criteria for diabetes diagnosis by 2010 ADA. There was still not, however, a guideline fit for Asia Pacific. To comprehensively evaluate the validity of 6.5% for diagnosing diabetes for Asian population, the systematic review and meta-analysis of cross sectional research on the accuracy of HbA_{1c} cut-off point in Asian population was performed.

Materials and methods: Electronic literature searching on MEDLINE, EMBASE and COCHRANE on the diagnostic accuracy of HbA_{1c} cut-off point published before February 2011 and handing search the reference lists of identified articles. Extract the data of optimal thresholds of HbA_{1c} for diabetes diagnosis posed by each study and calculate their medians. The summary sensitivities, specificities of each cut-off point described in these studies were calculated and the SROC curves were performed. Calculate the mean values

of HbA_{1c} and look for a cut-off point with highest specificity in confidence interval.

Results: Nine studies with uniform quality control published in English from 1989 to 2011 met the inclusion criteria. The total sample size was 18,543. The medians of optimal cut-off points posed by each study is 5.75%. The cut-off of 6.3% had the highest Youden's index was found in our study for Asian population with the corresponding sensitivity and specificity 69.0% (95%CI: 64.2%–73.5%) and 95.9% (95%CI: 95.3%–96.5%), respectively. The mean value of HbA_{1c} was 5.4±0.34%. In the confidence interval of mean±3SD, the cut-off point of 6.42% has the highest specificity. It was similar to 6.5% posed by ADA criteria. Meantime in our analysis, the cut-off point of 6.5% had higher specificity of 96.2% (95%CI: 95.8%–96.6%) and a higher area under SROC (0.8697) and its sensitivity was appropriate (41.8%, 95%CI: 38.6%–45.0%).

Conclusion: The cut-off point 6.42% with high specificity was similar to the ADA criteria. The HbA_{1c} cut-off point of 6.5% has a higher specificity and its sensitivity is not low either. Therefore we recommend that the HbA_{1c} cut-off point of 6.5% fits for diabetes diagnosis in Asian Pacific.

Optimal HbA_{1c} cut-off points conducted by 3 methods

Method/Value	Mediant of optimal cut-off point (%)	Cut-point with the highest Youden's index (%)	Mean value+3SD of HbA _{1c} (%)
value	5.75	6.3	6.42

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A comparison of determinants and progression rates of impaired glucose regulation (IGR) to type 2 diabetes using an oral glucose tolerance test (OGTT) and HbA_{1c} diagnostic criteria

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Background and aims: International organisations have included HbA_{1c} as a diagnostic criterion to detect T2DM. We investigated the progression rate of IGR (impaired glucose tolerance, IGT, and/ or impaired fasting glycaemia, IFG, using WHO 2011 criteria) to T2DM using HbA_{1c} or an OGTT during annual follow-up. Furthermore, we assessed the risk of developing diabetes with baseline HbA_{1c} categories and various risk factors.

Materials and methods: Analysis of the ADDITION-Leicester (UK) population based diabetes screening study from 2004–2010. Primary Care participants with IGR (n=909) detected at baseline were invited for an annual OGTT and HbA_{1c} measurement. Logistic regression analysis was used to produce adjusted odds ratios (OR) for progression to diabetes for risk factors in a stepwise procedure.

Results: For a median follow-up of 3 years (interquartile range 1–4), the progression rate to T2DM was 13.9% (95% CI 11.8–16.3%) and 14.3% (12.0–16.9%) using the OGTT and HbA_{1c}≥6.5% criteria respectively. However, only 5.5% had diabetes on both OGTT and an HbA_{1c}≥6.5%, suggesting major discordance between the two criteria. At follow-up, people with HbA_{1c}≥6.5% but without diabetes on OGTT were more likely to be of South Asian ethnicity (p<0.0001), have a lower BMI (p<0.0001), waist circumference (p<0.0001), waist:hip ratio (p=0.004) and diastolic BP (p=0.003) compared to individuals with T2DM on OGTT and HbA_{1c}<6.5%. 2-hour plasma glucose (2hpg, per 0.5mmol/l OR 1.3 (1.1–1.6)) and HbA_{1c} (per 0.5%, 1.6 (1.1–2.4)) were significant baseline predictors of progression from isolated-IGT to diabetes on OGTT, whilst age (per year, 0.95 (0.91–0.98)) produced an inverse association. For progression from isolated-IFG, fasting plasma glucose (FPG per 0.5mmol/l, 3.9 (1.1–14.1)), waist circumference (per cm, 1.1 (1.0–1.1)), 2hpg (1.6 (1.2–2.1) and history of CVD 6.2 (1.1–36.2) were significant predictors. For combined IGT/IFG, FPG (9.4 (2.4–37.0)), HbA_{1c} (1.7 (1.0–3.0)) and albumin: creatinine ratio (1.3 (1.0–1.7)) were significantly related to progression. Other anthropometric measures, sex, ethnicity, BP, lipids, family history of diabetes, current smoking status, fasting insulin, HOMA1-IR, leptin, adiponectin and Vitamin D were not significant. When HbA_{1c} criteria was applied at follow-up the significant baseline predictors of diabetes for isolated-IGT were HbA_{1c} (OR: 6.2 (3.3–11.6)); for isolated-IFG, HbA_{1c} (11.8 (2.9–48.6)) and South Asian ethnicity (5.1 (1.4–18.7)) and for combined IGT/IFG, HbA_{1c} (2.7 (1.0–7.1)) and FPG (5.0 (1.5–16.8)). Using a comparator of baseline HbA_{1c}<5.0%, the risk of progressing to HbA_{1c}≥6.5% over 3

years was significant (p<0.05) with baseline category HbA_{1c} 6.0–6.4% but not 5.5–5.9%, OR: 10.9 (3.1–37.7) and 3.3 (0.9–11.8).

Conclusion: Progression rates for people with IGR to diabetes was similar using an OGTT or HbA_{1c}, suggesting re-screening IGR using either test in clinical practice is feasible, however they detect different people with different clinical characteristics. The most consistent determinants of IGR progression to diabetes were glucose blood tests, with a limited scope for non-invasive measures, basic demographics and biomarkers. The risk of developing diabetes on HbA_{1c} criteria was significant only for people with HbA_{1c}≥6.0%.

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5-year temporal change in HbA_{1c} and its risk factors in the Japanese general population

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Background: Because of aging and obesity, the number of people with diabetes (DM) is increasing rapidly worldwide. In Japan, the prevalence of DM has been monitored based on A1C ≥6.5% since 1997 and has been repeated with a 5-year interval as the Japan National Diabetes Survey. There are very few data available on temporal trends in the distribution of A1C at population level and on factors explaining temporal changes. The aim of this study is to examine 5-year temporal change in A1C from 1997 to 2002 and risk factors for A1C in Japan, by using cross-sectional data from the Japan National Diabetes Survey conducted in 1997 and 2002.

Methods: The study comprises 4,439 men and 6,716 women aged 20 years and older. The demographic and clinical data and proportions of people classified by five groups on A1C (<5.6, 5.6–5.9, 6.0–6.4, ≥6.5%, medicated DM) were compared between two surveys years. The step-wise multivariate regression model with age (10 years), body mass index (BMI) (1 kg/m²), systolic blood pressure (SBP) (10 mmHg), total cholesterol (TC) (1 mmol/l), survey year (2002 vs. 1997), antihypertensive medication, regular exercise habits, step/day (1,000 steps/day), current smoking status, and current alcohol habits as independent variables was fitted to identify risk factors for HbA_{1c} in people without medicated DM.

Results: In men, mean age, BMI and the proportion of people on antihypertensive medications were significantly higher, while mean step/day and the proportion of current smokers were significantly lower in 2002 than 1997. In women, mean age, TC and the proportion of people having regular exercise habits were significantly higher in 2002 than 1997. The crude proportion for men and women with A1C ≥6.5% and for men with medicated DM did not change, but the crude proportion for men and women with A1C 5.6–6.4% was significantly increased for 5-years (men: 23.9→34.6%, women: 28.3→40.1%, p<0.001). The median A1C shifted from 5.3 to 5.5% for 5 years in men and women without medicated DM. When adjusting for age, mean A1C was significantly higher in 2002 than 1997 in the group of men and women with BMI <30 kg/m². When adjusting for BMI, mean A1C was significantly higher in 2002 than 1997 in the group of men <40 years old and women <50 years old. Table 1 shows beta coefficient and its standard error of risk factors for 1% increase of A1C. The survey year was identified as an independent risk factor for increase of A1C as well as established risk factors of DM.

Conclusions: The median A1C increased by 0.2% from 1997 and 2002 in Japan. This may be partly due to an elevation of A1C in young-middle aged or non-obese individuals. However, the temporal increase of A1C in Japan was not fully explained by established risk factors of DM in this study.

Multivariate regression analysis for 1% increase in A1C in people without medicated DM

	Men			Women		
	B	SE	P-value	B	SE	P-value
Age (10 years)	0.108	0.007	<0.001	0.063	0.005	<0.001
BMI (1 kg/m ²)	0.032	0.003	<0.001	0.028	0.002	<0.001
SBP (10 mmHg)	0.012	0.006	0.046	0.021	0.004	<0.001
TC (1 mmol/l)	0.084	0.012	<0.001	0.057	0.008	<0.001
Current smoking (yes vs. no)	0.066	0.021	0.002			
Current alcohol (yes vs. no)	-0.077	0.021	<0.001	-0.084	0.025	0.001
Step/day (1000 steps)	0.005	0.002	0.044			
Survey year (2002 vs. 1997)	0.151	0.021	<0.001	0.140	0.014	<0.001
R square	0.123			0.148		
Adjusted R square	0.121			0.147		

Excluded variables were regular exercise habits and antihypertensive medication for men and women, step/day and current smoking habits for women.

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Predictive value of HbA_{1c} for the occurrence of diabetes in normoglycaemic people: the REDIA cohort - Reunion Island 1999-2001 / 2006-2009

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Background and aims: The glycated hemoglobin (HbA_{1c}) is a parameter commonly used for glycaemic monitoring in diabetic patients. Recently it has been proposed as a diagnostic criterion for diabetes at the threshold $\geq 6.5\%$. Aims: to verify that among non diabetic normo-glycaemic adults, the gradient of increasing HbA_{1c} values below 6.0% is predictive of diabetes or glucose abnormalities detected several years after.

Materials and methods: Among 4610 persons aged 18-69, residing in three cities of Reunion Island and recruited in the REDIA survey on diabetes in 1999-2001, 3096 participated in a follow-up cohort in 2006-2009. At the inclusion, 2025 persons unknown as diabetics were diagnosed normo-glycaemic by capillary blood glucose test (<1.00 g / l fasting / 1.40 g / l non-fasting) and capillary HbA_{1c} test ($<6.0\%$). The predictive value of HbA_{1c} on the onset of diabetes (treated or detected) and glycemia (HbA_{1c} $\geq 6.0\%$) was assessed by: 1) the area under the ROC curve (AUC), 2) the relative risks estimated by modified Poisson regression models.

Results: Participants were followed for 7.4 years (median). Whatever the glycaemic event considered, the discriminatory value of the initial HbA_{1c} was satisfactory (AUC ~ 0.75). After taking into account gender, age, BMI, hypertension, family history of diabetes and monitoring delay, the risk of abnormal glucose control (HbA_{1c} $\geq 6.0\%$) increased exponentially with Initial HbA_{1c} ($p < 0.0001$), whereas at the threshold of 6.5% the risk increased only from 5.4-5.9% ($p < 0.0001$). In people with no direct family history of diabetes, the risk of diabetes increased linearly with HbA_{1c} ($p < 0.0001$), whereas among those reporting a direct family history of diabetes, the risk increased only from 5.4-5.9% ($p = 0.0004$).

Conclusion: Our populational study in Reunion island shows that the probability of incident type 2 diabetes increases with HbA_{1c} measured at inclusion, even at an initial risk level lower than that usually seen in the general population (ie $\geq 6.0\%$) and during a relatively short period (< 10 years).

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Changes in glucose and HbA_{1c} prior to the diagnosis of diabetes: a 10-year follow-up of the Hoorn Study

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Background and aims: It is unknown whether early changes in glucose levels prior to the diagnosis of diabetes, as shown in previous studies, are accompanied by changes in A1c. To evaluate this, we compared changes in fasting glucose, postload glucose and A1c levels over a 10-year period in participants of the Hoorn Study who did and did not develop diabetes.

Materials and methods: For the analysis we used data of 565 non-diabetic participants of the Hoorn Study between 50-75 years of age who had an OGTT and A1c determination at baseline (1989), first (1996) and second (2000) follow-up. Diabetes was diagnosed at follow-up based on fasting glucose ≥ 7.0 mmol/L, and/or postload glucose ≥ 11.1 mmol/L, and/or A1c $\geq 6.5\%$, and/or use of diabetes medication. Three groups were created: participants without diabetes during follow-up ($n = 418$), participants with incident diabetes in 1996 ($n = 99$) and participants with incident diabetes in 2000 ($n = 48$). Curves were plotted to compare the time course of glycemic measures in the three groups. To test whether the time-dependent changes in glycemic measures differed between participants with diabetes and those without diabetes during follow-up, linear mixed models were used.

Results: In Figure 1, the time course of glycemic measures in participants who did and did not develop diabetes is shown. Participants who developed diabetes already had higher fasting and postload glucose levels and A1c as compared to those who did not develop diabetes. In participants with incident diabetes in 1996 (top lines in Figure 1), mean fasting glucose, A1c and postload glucose increased significantly from 1989 to 1996 as compared to participants without diabetes (bottom lines in Figure 1). After the diagnosis in 1996, all glycemic measures stabilized towards 2000. Mean levels of fasting glucose and postload glucose in participants with incident diabetes in 2000 (dotted lines in Figure 1) increased gradually between 1989 and 1996, but not significantly different from those without diabetes ($p > 0.10$). In contrast, A1c levels already showed a significant increase in future diabetes patients between 1989 and 1996 compared to those without diabetes ($p < 0.001$). Between 1996 and 2000, rapid increases in fasting glucose, and especially postload glucose and A1c were seen in those who developed diabetes in 2000 as compared to those without diabetes (all $p < 0.001$).

Conclusion: Future diabetes patients already have increased levels of glucose and A1c 10 years before the diagnosis of diabetes. These levels rapidly increase a few years before the diagnosis, with an earlier increase in A1c as compared to glucose.

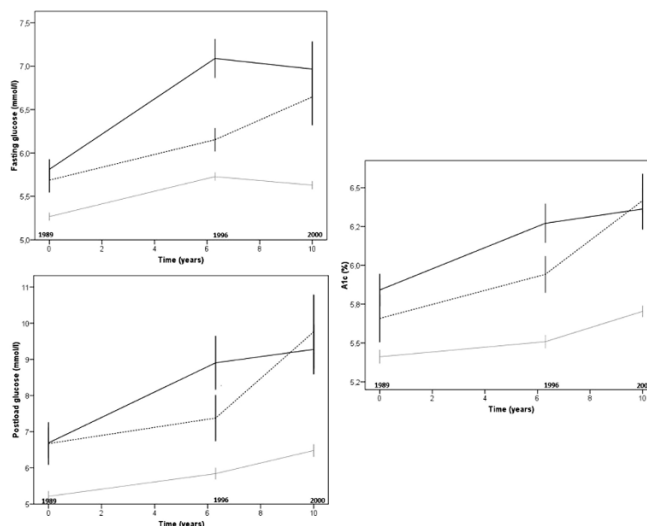


Figure 1. Changes in fasting and postload glucose and A1c in those with incident diabetes in 1996 (top lines, N=99), those with incident diabetes in 2000 (dotted lines, N=48) and those who did not develop diabetes during 10 years of follow-up (bottom lines, N=418). Data are presented as the mean and 95% confidence intervals at all time points.

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Diagnosis of diabetes with HbA_{1c} value screens different population between genders in terms of glucose tolerance and insulin secretory reaction

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Background and aims: Diagnostic criterion of diabetes mellitus recommended by WHO or ADA adopted HbA_{1c} as well as the plasma glucose at OGTT. We have characterized the diagnosis of diabetes using HbA_{1c} from the view point of glucose tolerance and insulin secretion observed at OGTT.

Methods: In order to evaluate the validity of diagnosing of diabetes with HbA_{1c}, cross-sectional analysis was performed in 3,142 Japanese subjects (men 66 %) with the examination of 75g OGTT and HbA_{1c}. Serum IRI was also measured in OGTT. These subjects were divided into 4 groups, categorized as low risk (HbA_{1c}<6.0: A), high risk (6.0≤HbA_{1c}<6.5: B), mild diabetes (6.5≤HbA_{1c}<6.9: C) and overt diabetes (HbA_{1c}≥6.9: D), depending on their HbA_{1c} value and the results of 75g OGTT were compared between genders in each categories. In Japan, cases with more than 6.9% of HbA_{1c} were formally diagnosed as diabetes. Statistical analysis was conducted with two-way ANOVA and multiple regression analysis using commercially available computer software, JMP 7 (SAS Institute, Cary, NC, USA).

Result and discussion: We recognized significant and unignorable differences of plasma glucose level at each time point in OGTT between genders by up to 32 mg/dL in group C. Regarding FPG (mg/dL) and FIRI (μU/ml) women showed significantly lower level than men by 4.9 and 1.0 in group A, by 7.7 and 1.5 in group B, respectively. In group C, only FPG was significantly lower in women by 5.6. As shown in table, 2h-post challenge plasma glucose (2hPG) level showed significant larger difference between genders. Insulin secretory response was also explored at OGTT. Significant lower 2h-post challenge IRI was observed in women by 5.5 in group A, and by 7.4 in group B. HOMA-IR showed significant lower value in women in group A and B, which might cause the gender difference of glucose profile; while this wasn't so evident in group C or D. As for HOMA-β, in contrast, women had significant higher value in group C. Multiple regression analysis explored some indices decisive of 2hr postchallenge glucose. Both HOMA-IR and HOMA-β contributed significantly to 2hPG in all groups of A, B, C and D, with HOMA-IR positively and HOMA-β negatively. Insulinogenic index at 1 hour (1hr I.I) affected significantly and negatively to 2hPG in groups B, C and D although not significantly between genders. To be female was negative factor to determine 2hPG in group A, B, and C.

Conclusion: A significant gender difference was recognized in the glucose and insulin level at OGTT, when glucose tolerance was categorized with HbA_{1c} value. These data suggest diagnosis of diabetes with OGTT or HbA_{1c} screens different population between genders, which results in over-diagnosis of the stages of glucose intolerance in women. Data are shown in the form of mean ± SD, *: <0.05 **: < 0.01 ***: <0.0001, HOMA-IR: Homeostasis model assessment-Insulin Resistance, HOMA-β: Homeostasis model assessment beta cell model, 1h I.I: [(1hrIRI)-(FIRI)]/[1(hPG)-(FPG)]

Indices derived from OGTT

	A (HbA _{1c} < 6.0)		B (6.0 ≤ HbA _{1c} < 6.5)		C (6.5 ≤ HbA _{1c} < 6.9)		D (HbA _{1c} ≥ 6.9)	
	men	women	men	women	men	women	men	women
2hPG (md/dl)	125.2±0.8	115.6±1.1***	155.7±2.0	134.7±3.0***	206.1±5.7	173.3±11.3*	290.7±6.5	309.8±21.1
HOMA-IR	1.61±0.02	1.30±0.03***	2.04±0.05***	1.52±0.08***	2.32±0.13	2.65±0.27	3.51±0.20	2.96±0.55
HOMA-β	71.0±0.8	71.2±1.2	61.6±1.5	59.1±2.1	50.4±2.7	67.0±5.6**	34.3±6.5	30.9±17.6
1h I.I	0.75±0.14	0.95±0.18	0.59±0.07	0.80±0.10	0.40±0.06	0.41±0.13	0.19±0.04	0.15±0.14

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Haemoglobin susceptibility to glycosylation may partly explain discordance between HbA_{1c} measurement and oral glucose tolerance test to diagnose dysglycaemia

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Background and aims: The aim was to assess whether the poor consistency for dysglycemia diagnosis of HbA_{1c} and oral glucose tolerance test (OGTT) may be explained by hemoglobin glycosylation susceptibility (GS).

Materials and methods: We included 1033 consecutive overweighted or obese patients without renal failure, abnormal thyroid function or known diabetes, and having had fructosamine (glycosylated proteins; colorimetry) and HbA_{1c} (turbidimetric immuno-assay) measurement and an OGTT. Predicted HbA_{1c} from albumin corrected-fructosamine (Alb-F) was calculated from the following regression: predicted HbA_{1c} = 0.002 x Alb-F + 4.56 (r=0.185, p<0.0001). Three groups were determined: "High-GS" / "Low-GS" (5% / 5% of patients having the greatest / lowest measured HbA_{1c} as compared to predicted HbA_{1c}) and "Intermediate-GS" (90% remaining patients).

Results: On the basis of the OGTT and HbA_{1c} measurement respectively, 267 (25.8%) and 443 (42.8%) patients had intermediate hyperglycemia and 66 (6.4%) and 95 patients (9.2%) had diabetes (American Diabetes Association criteria 2011). The results were inconsistent for dysglycemia diagnosis in 31.1% and for diabetes diagnosis in 19.6% of the patients. In the "High-GS" subjects, the proportion of false positive results of HbA_{1c} as compared with OGTT was 59.6% for diabetes and 30.8% for dysglycemia; versus 3.8 and 32.5% in the "Intermediate-GS" and 0 and 0% in the "Low-GS" patients (p<0.0001). In mirror, the "Low-GS" subjects had more false negative results of HbA_{1c} as compared with OGTT. Multivariate analysis showed that body mass index (odds ratio 1.06 [95% confidence interval 1.01-1.12], p=0.015), fasting plasma glucose (per mmol/l, OR 1.83 [1.22-2.75], p=0.003) and 2h-plasma glucose (OR 1.17 [1.02-1.34] 0.023) were independently associated with high-GS.

Conclusion: Discordant results of HbA_{1c} and OGTT for diagnosing dysglycemia may be partly explained by hemoglobin GS, which increases with higher body mass index and glucose values even in subnormal range.

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Determinants of HbA_{1c} in non-diabetic Dutch adults: genes, environment and their interactions in the LifeLines cohort study
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Background and aims: HbA_{1c} is associated with cardiovascular risk in persons without diabetes, and its use has been recommended for diagnosing diabetes. Therefore, it is important to get better insight in determinants of HbA_{1c}. In this study we investigate the effect of environmental factors, genetic loci and gene-environment interactions on HbA_{1c} in non-diabetic adults.

Materials and methods: BMI, waist circumference, HbA_{1c}, fasting plasma glucose (FPG) and erythrocyte indices were measured in 2,921 non-diabetic adults participating in the population-based LifeLines study. Data on current smoking and alcohol consumption were collected by questionnaires. Genome-wide genotyping was performed, 12 previously identified SNPs were selected for replication and categorized in “glycaemic” and “non-glycaemic” SNPs according to the presumed way they act on HbA_{1c}. Genetic risk scores (GRSs) were calculated by adding up the weighted effect of HbA_{1c}-increasing alleles.

Results: Age, gender, BMI, FPG, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), current smoking and alcohol consumption were independent predictors of HbA_{1c}, together explaining 26.2% of the variance in HbA_{1c}, with FPG only contributing 10.9%. We replicated three of the previously identified SNPs, namely rs1402837, rs4737009 and rs1046896 and the GRSs were also independently associated with HbA_{1c}. We found a smaller effect of the “non-glycaemic GRS” in females compared to males and an attenuation of the effect of the “glycaemic GRS” with increasing BMI.

Conclusion: Our results suggest that a substantial part of HbA_{1c} is determined by non-glycaemic factors, raising serious questions about the use of HbA_{1c} as a diagnostic test for diabetes.

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PS 009 New diagnostic tools

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Association of skin intrinsic fluorescence with cumulative glycaemic exposure in the DCCT/EDIC study

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Background and aims: We have previously demonstrated that dermal advanced glycation end products (AGEs) are associated with microvascular complications in a subset of the Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications (DCCT/ EDIC) cohort. Other studies have shown a correlation of AGEs with skin intrinsic fluorescence (SIF). In addition, DCCT/EDIC has shown that glycemic exposure as expressed by overall mean HbA_{1c} is strongly associated with microvascular complications. We thus performed a substudy to evaluate the relationship of SIF and glycemic exposure.

Materials and methods: SIF was measured noninvasively on the volar forearm of 1,080 participants. SIF was quantified using the SCOUT device after an average DCCT/EDIC follow-up of 23.5 years. SIF had a log normal distribution, thus was log transformed for analysis. Glycemic exposure was expressed as time weighted mean HbA_{1c}.

Results: The proportion of variation (R²) in log SIF explained by mean HbA_{1c} (Table 1) over various periods was low (< 9%), but statistically significant for all measures of mean HbA_{1c}, except ‘DCCT only’ for the intensive therapy group. The correlation of the DCCT mean HbA_{1c} with SIF was less than that for the EDIC mean HbA_{1c}; the overall mean HbA_{1c} had the strongest correlation.

Table 1	Combined	INT	CONV	INT vs. CONV
N	1,080	561	519	
Mean HbA_{1c}	Unadjusted R ² with log SIF			p-value ~
Time Span				
Last 5 yrs EDIC	0.030	0.024	0.037	0.4676
Last 10 yrs EDIC	0.041	0.034	0.049	0.3864
EDIC only	0.056	0.048	0.067	0.2541
DCCT only	0.011	0.005 #	0.028	0.3392
DCCT+EDIC	0.053	0.038	0.074	0.1192
Overall *	0.065	0.050	0.083	0.1890

* Overall HbA_{1c} is calculated by summing [DCCT/EDIC eligibility HbA_{1c} x duration of diabetes at study baseline], [DCCT mean HbA_{1c} x years of follow-up in DCCT], [EDIC mean HbA_{1c} x years of follow-up in EDIC].

P=0.0993. All other correlations are significant at p=0.01.

~ P-value for interaction in a model with treatment group, HbA_{1c}, and their interaction

The R² in the intensive (INT) group was slightly less than that in the conventional (CONV) group for the EDIC periods but substantially less for the DCCT period (Table 1). Log SIF was not significantly different between the DCCT intensive and conventional treatment groups after adjusting for the significant effects of skin tone as measured by the SCOUT device, age, smoking status, and clinic latitude (above/below 37°) (geometric means 22.2 AU vs. 22.3 AU, p=0.67). In the combined cohort, the association of overall mean HbA_{1c} with log SIF was 0.068 (semipartial R²) after adjustment for skin tone, age, smoking status, and clinic latitude. The overall model explained 30% of the variation in log SIF. The model for men explained a higher proportion of the variation in log SIF compared to that for women (model R² 37% vs. 23%). SIF was associated with overall mean HbA_{1c}, subject age, smoking status, skin tone, and clinic latitude among both males and females. Overall, mean HbA_{1c} and subject age had a stronger association with log SIF among males than females.

Conclusion: Over the 23.5 years of DCCT/EDIC, glycemic exposure as represented by the overall mean HbA_{1c} along with subject age and smoking status, explained the most skin intrinsic fluorescence variance in both the combined and gender stratified analysis.

Clinical Trial Registration Number: NCT00360893

Supported by: VeraLight, Inc.

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Non-invasive skin fluorescence age trends in subjects with normal health are gender dependent

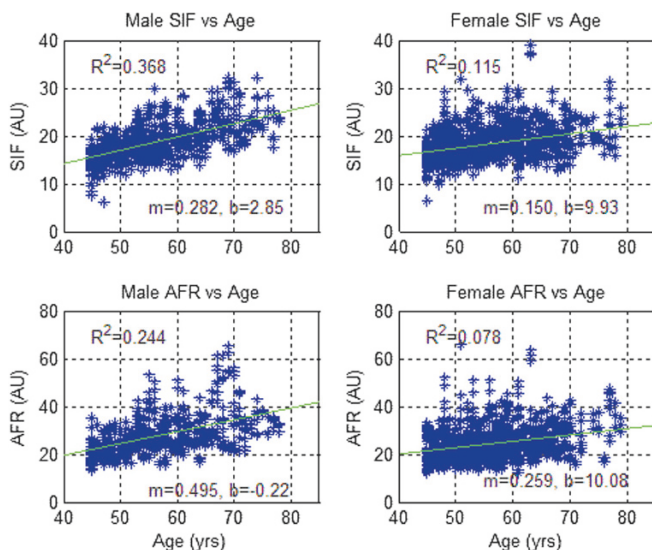
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Background and aims: Non-invasive skin intrinsic fluorescence (SIF) and the autofluorescence ratio (AFR) are both associated with the accumulation of advanced glycation endproducts (AGEs) and have been used to assess risk of diabetes complications. SIF has also been investigated as a screening test for pre-diabetes and type 2 diabetes. AGEs have been shown to increase as a function of age in normal health and at a faster rate in subjects with diabetes. We studied 777 subjects in normal health to estimate the change in SIF and AFR as a function of age and gender.

Materials and methods: Subjects without a pre-existing diagnosis of diabetes were recruited for a non-invasive diabetes screening study involving two visits to the clinic. The first visit required an overnight fast (> 8 hrs), while the second visit was in a random fasting state. During the first visit, subjects gave informed consent, answered a health and demographics questionnaire and had blood pressure measured. Fasting plasma glucose (FPG), A1c and 2 hour post challenge glucose (2HPG) after a 75 gram oral glucose tolerance test were also measured. At each visit, SIF and AFR were measured on the left volar forearm near the elbow using a SCOUT device that quantifies both skin reflectance and fluorescence in the 360 to 660 nm spectral range. Skin fluorescence was excited by a 375 nm LED. A white LED was used to measure skin reflectance. SIF and AFR were calculated over 435 to 655 nm using algorithms described in the literature. Subjects with skin reflectance > 1 absorbance unit were excluded from the AFR age trend analysis because AFR is overestimated in these cases. Least-squares linear fits of SIF and AFR versus age were calculated by gender for subjects in normal health that were 45 to 80 years old and not current smokers. Normal health was defined as FPG < 6.1 mmol and 2HPG < 7.8 mmol and A1c < 6.0% and no renal compromise, cardiovascular disease or hypertension.

Results: 273 men and 504 women satisfied the normal health criteria. Ethnic distribution was 594 white, 80 Hispanic, 86 African American and 17 other. The median age was 54 ± 7.7 years and median A1c was $5.5 \pm 0.3\%$. Figure 1 shows the correlations (R^2), slopes (m), and intercepts (b) between SIF and AFR vs. age for each gender; all correlations were significant ($p < 0.0001$). For both SIF and AFR, the male slope was 1.9 times greater than the female slope, while females had a larger intercept. To test if the age and gender interaction is significant, SIF and AFR linear regression models for the entire cohort were constructed using age, gender and age x gender as the independent variables. The age x gender interaction was significant ($p < 0.0001$) for SIF and AFR.

Conclusion: For subjects in normal health, SIF and AFR demonstrate significant, gender dependent trends with increasing age. Non-invasive screening for pre-diabetes and type 2 diabetes or assessing risk of diabetes complications with SIF or AFR may be confounded by these underlying trends and performance improvements may be possible if they are accounted for.



Clinical Trial Registration Number: NCT00614783

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Which factors determine performance of skin autofluorescence-based decision tree for detection of impaired glucose tolerance and diabetes?

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Background and aims: Noninvasive skin autofluorescence (AF) is a marker of tissue accumulation of advanced glycation endproducts (AGE) which has been proposed as a carrier of glycometabolic memory. Skin AF has been reported to be superior to fasting plasma glucose (FPG) and HbA1c for the detection of impaired glucose tolerance IGT and diabetes in studies in naive persons, and in patients at intermediate-high risk of diabetes. Further improvement in the diagnostic performance of skin AF has been obtained in a decision tree (Diab-Spot), incorporating the items calendar age, BMI, number of 1st degree relatives with diabetes, and conditional questions on recent hospital admissions and on kidney disease. We analysed the relative contributions of these and other clinical items on the misclassification rates of this decision tree in persons at intermediate high risk of IGT or diabetes.

Materials and methods: Diagnostic performance (for IGT/suspicion diabetes, and for diabetes, respectively) and misclassification rates/numbers were assessed against WHO-GTT cutoff values in a 2-hour GTT in persons with 2 metabolic syndrome criteria. This was done for age-corrected skin AF alone, and using factor analysis for addition of the further items in Diab-Spot. Results were compared to those for glycemic criteria FPG and HbA1c (using WHO and IEC 2009 cutoff values, respectively).

Results: 218 persons, age 56 yr (33 <45, 23 >70), 128M/90 F, s-creatinine 82 (40-166) $\mu\text{mol/L}$, participated, 93 with previous cardiovascular events. With GTT-based WHO criteria 28 had diabetes, 46 IGT, 41 IFG, 103 normal glucose tolerance (NGT). With HbA1c-based IEC-2009 criteria, 13 had diabetes, 87 suspicion, and 117 NGT. Using AF alone to detect GTT-based diabetes/IGT, 57 were misclassified (23 false positives, 34 false negatives), with 68.5% sensitivity (S) and 86% specificity (SP). Factor analysis revealed that addition of BMI and family history contributed most in improving S, and questions on hospital admissions SP, to overall 83% and 90%, respectively. Items on sex, smoking, known kidney disease, use of antihypertensives did not add significantly. For FPG, using IEC criteria for DM (suspicion), S and SP were 64 and 83%, respectively, for HbA1c 80 and 75%.

Conclusion: Skin AF-based decision model has superior performance compared with FPG or HbA1c in intermediate risk persons to detect diabetes/IGT. Other relevant factors include age, BMI, family history. Noninvasive skin AF is promising for diabetes screening and further supported by its previously proven additive value in predicting diabetic complications.

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Utility of glycated albumin for the diagnosis of diabetes mellitus in a Japanese population study: results from the Kyushu and Okinawa Population Study (KOPS)

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Background and aims: Glycated albumin (GA) is a measure of the mean plasma glucose level over approximately 2–3 weeks. We determined reference values for GA, and assessed the utility of GA in the diagnosis of diabetes mellitus (DM) in the general population.

Materials and methods: We studied 1,575 men and women (mean age, 49.9 years; range, 26–78 years) participating in a periodic health examination in a suburban Japanese town. Glycohemoglobin A1c (HbA1c) and fasting plasma concentrations of glucose (FPG), and GA were measured. Participants with FPG 7.0 or more mmol/L or HbA1c 6.5 % or more were diagnosed as having DM. The GA assay has the benefits of being well standardized, with excellent coefficients of variation and can be run using high throughput automated analyzers. Moreover the assay does not require whole blood.

Results: GA levels were significantly correlated with HbA1c level ($r = 0.766$, $P < 0.001$) and FPG ($r = 0.706$, $P < 0.001$). The presence of DM was significantly

higher in participants with GA levels between 15.0 and 15.9 % than in those with GA less than 14 % ($P = 0.037$), and was markedly increased in those with GA values more than 16 %. Receiver operating characteristic curve analysis indicated that a GA level of 15.5 % or more was optimal for the diagnosis of DM, with a sensitivity of 83.3% and a specificity of 83.3%.

Conclusion: We recommend a GA value 15.5 or more % as an optimal cut-point for the diagnosis of DM in the general population.

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The relationship between plasma glycated albumin level and atherosclerosis: results from the Kyushu and Okinawa population study (KOPS)

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Background and aims: Glycated albumin (GA) is a recognized indicator of glycemic control. This study was done to assess the hypothesis that GA can serve as well as glycohemoglobin A1c (HbA1c) as a risk marker of atherosclerosis.

Material and methods: HbA1c and fasting plasma concentrations of GA and high-sensitive C-reactive protein (hs-CRP) were measured for 1,575 residents (mean age, 49.9 years; range, 26–78 years) of a suburban Japanese town. Carotid artery intima-media thickness (IMT) was measured by ultrasound for each subject. Obesity was defined as body mass index $> 25 \text{ kg/m}^2$.

Results: The hs-CRP and max-IMT of participants with GA $> 19 \%$ were significantly higher than the values of those with GA $< 14 \%$ (hs-CRP: 0.121 ± 0.148 vs. $0.060 \pm 0.101 \text{ mg/L}$, $P < 0.01$; max-IMT 1.109 ± 0.562 vs. $0.757 \pm 0.317 \text{ mm}$, $P = 7.0 \%$ were significantly higher than the values of those with HbA1c $< 5.0 \%$ (hs-CRP: 0.154 ± 0.192 vs. $0.040 \pm 0.083 \text{ mg/L}$, $P < 0.01$; max-IMT 1.094 ± 0.559 vs. $0.702 \pm 0.268 \text{ mm}$, $P = 16 \%$ were significantly higher in than the values of those with GA $< 16 \%$. Among subjects with GA of 16 to 19 %, the hs-CRP and max IMT were significantly higher for obese subjects than for non-obese subjects.

Conclusions: Similar to HbA1c, a high plasma GA level was associated with inflammation and atherosclerosis. GA may be useful as a risk marker for atherosclerosis. Persons with a high GA level are at risk of developing atherosclerosis, especially if they are obese.

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Characteristics of 24-hour glycaemic excursions revealed by continuous glucose monitoring in subjects with normal glucose tolerance and impaired glucose tolerance

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Background and aims: Continuous glycemic monitoring provides much more information about shifting glycemic levels throughout the day. In this study, continuous glucose monitoring system (CGMS) was used to further investigate the characteristics of 24-hour glycemic excursions in subjects with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT).

Materials and methods: The glycemic excursions and tendency of 51 newly diagnosed IGT subjects (aged 59 (31–80) yrs, 25M/26F) and 41 NGT subjects (aged 42 (25–69) yrs, 21M/20F) were measured by the CGMS for 3 days. The CGMS took the glycemic measurements for a total of 288 values every 24 hours.

Results: IGT group in comparison with NGT group showed significantly higher values (means \pm SEM) of body mass index ($25.3 \pm 0.5 \text{ kg/m}^2$ vs $22.8 \pm 0.4 \text{ kg/m}^2$), systolic blood pressure ($130 \pm 3 \text{ mmHg}$ vs $119 \pm 2 \text{ mmHg}$), HbA_{1c} ($6.13 \pm 0.08 \%$ vs $5.53 \pm 0.09 \%$) and triglyceride ($1.80 \pm 0.20 \text{ mmol/l}$ vs $1.15 \pm 0.13 \text{ mmol/l}$) (all $P < 0.01$). The profiles of the CGMS showed: There were significant differences in the average glycemic level ($5.31 \pm 0.08 \text{ mmol/l}$

vs $6.48 \pm 0.11 \text{ mmol/l}$), the standard deviation of 24-hour glycemic values ($0.88 \pm 0.04 \text{ mmol/l}$ vs $1.48 \pm 0.08 \text{ mmol/l}$), and the maximal amplitude of glycemic excursions ($3.91 \pm 0.25 \text{ mmol/l}$ vs $7.10 \pm 0.37 \text{ mmol/l}$) between NGT group and IGT group ($P < 0.01$). 60.8% diurnal glycemic peaks occurred in 6am–10am period, but no glycemic peaks were observed during 9pm–5am period in IGT group. The values of postprandial glycemic peaks, time to postprandial glycemic peaks, the postprandial amplitude of glycemic excursions and the area under the curve (AUC) of postprandial glucose in IGT group were $10.17 \pm 0.30 \text{ mmol/l}$, $74.6 \pm 4.5 \text{ min}$, $4.13 \pm 0.27 \text{ mmol/l}$, $0.27 \pm 0.02 \text{ mmol/l-24h}$ in breakfast; $9.15 \pm 0.30 \text{ mmol/l}$, $93.0 \pm 4.9 \text{ min}$, $3.76 \pm 0.32 \text{ mmol/l}$, $0.37 \pm 0.03 \text{ mmol/l-24h}$ in lunch; $8.96 \pm 0.27 \text{ mmol/l}$, $86.6 \pm 4.9 \text{ min}$, $3.31 \pm 0.26 \text{ mmol/l}$, $0.33 \pm 0.03 \text{ mmol/l-24h}$ in supper; respectively, which were all significantly higher than those in NGT group (all $P < 0.01$). The glycemic peak of post-breakfast was higher than those of post-lunch and supper, but the AUC of post-lunch glucose was higher than those of post-breakfast and supper.

Conclusion: Continuous glucose monitoring offers advantages to revealing more details about the glycemic excursions and tendency throughout the day in IGT subjects. The glycemic profiles from the CGMS have a valuable role in identifying the characteristics of glycemic excursions and making the optimal treatment decisions in IGT.

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Beyond the morphology of the glucose curve following an oral glucose tolerance test in obese youth

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Background and aims: The morphology of the glucose curve following an oral glucose tolerance test may predict the risk of altered glucose metabolism and harbor metabolic information not captured by the level of glycaemia alone. Aim of the present study was to describe any association between morphology of the glucose curve during the OGTT and insulin action, insulin secretion and glucose tolerance in obese children and adolescents.

Materials and methods: OGTT data of 553 young obese patients were analyzed. Subjects were divided in groups based on morphology of the glucose curve (i.e., monophasic, biphasic, triphasic and upward monotonous curves). Insulin sensitivity/resistance was estimated by the homeostasis model assessment (HOMA-IR), the insulin sensitivity (ISI), the muscular insulin sensitivity (MISI) and the hepatic insulin resistance (HIRI) indexes, and the oral glucose insulin sensitivity (OGIS). Insulin secretion was estimated by the insulinogenic index (IGI). Areas under glucose (AUC_G) and insulin (AUC_I) curves were computed.

Results: The prevalent morphology of the glucose curve was monophasic ($N=285$, 54%). Monophasic morphology was associated with the highest concentration of 1-hour plasma glucose (1HPG, $P < 0.0001$) and AUC_G ($P < 0.0001$). Individuals with biphasic morphology had lower OGIS ($P=0.01$) and greater AUC_I ($P < 0.0001$) than those observed in subjects with monophasic morphology. Triphasic morphology was associated with the highest values of HIRI ($P=0.02$) and IGI ($P=0.007$). By combining either morphologies of the glucose curve and time of glucose peaking or morphologies of the insulin curve, a deeper characterization of different phenotypes of glucose metabolism emerged.

Conclusion: Morphologies of the glucose curves seem reflecting different metabolic phenotypes of insulin action and insulin secretion in the class of normo-tolerant individuals. Findings of this study may deserve validation in a cohort study, in which glucose metabolism would be estimated by using gold-standard techniques.

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The assessment of 1-h plasma glucose during an oral glucose tolerance test in a group of Romanian subjectsR. Dinu¹, S. Popa¹, M. Mota¹, E. Mota², M. Cruce³;¹Diabetes, Nutrition, Metabolic Diseases ²Nephrology, ³Molecular and Cell Biology, University of Medicine and Pharmacy Craiova, Romania.

Background and aims: Type 2 diabetes mellitus (DM) is one of the most common forms of chronic disease globally. In 2010, it was estimated that 285 million people worldwide have diabetes. Many important clinical trials demonstrated that early diagnosis and treatment can prevent or delay the onset of diabetes complications. The golden criterion for the diagnosis of DM remains the oral glucose tolerance test (OGTT) with 75g glucose. If the diagnosis is based upon 2-h plasma glucose value, the 1-h value is taken in consideration only for the diagnosis of gestational diabetes but not for DM. The aim of our study was to evaluate the 1-h plasma glucose value during OGTT in the diagnosis of diabetes.

Materials and methods: The study comprised of 412 subjects that performed a 2-h OGTT. According to the glucose values recorded during OGTT, the subjects were divided in 5 groups: without DM, IFG, IGT, IFG+IGT, DM. The statistical analysis was performed using SPSS 17.0 for Windows and the p-value under 0.05 was considered significant.

Results: The 1-h glucose value for each group was 163.34±38.16, 164.34±42.29, 184.83±46.86, 201.03±39.17 and 247.03±64.36 (p<0.001). The post-hoc analysis revealed that the 1-h glucose value for the subgroup without DM and, respectively, with DM were significantly different from the other subgroups. Using the receiver operating characteristic (ROC) curve, we tried to determine the cut-off value for the 1-h glucose value in diagnosing diabetes. The area under ROC curve was 0.853 (95%CI=0.806-0.897). The value of 215.5 mg/dl (sensitivity: 69.1%, specificity: 86.1%) was the point on the ROC curve with maximum Youden index and with shortest distance value from the point (0,1). When comparing the subjects without DM with all the subjects with impaired glucose metabolism, 168.5 mg/dl (sensitivity: 73.8%, specificity: 77.2%) had the maximum Youden index and 179.5 mg/dl (sensitivity: 63.8%, specificity: 87.8%) had the shortest distance value from the point (0,1).

Conclusion: These data indicate that the 1-h glucose value is a good predictor for diabetes. The value from the subjects without DM is significantly different from the value of the subjects with impaired glucose metabolism. The value of 215.5mg% can be considered a cut-off for the diagnosis of diabetes. Further research is needed to determine the role of the 1-h glucose value during the OGTT.

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Genome-wide association study on obesity in patients with type 1 diabetesE. Fagerholm^{1,2}, N. Sandholm^{1,2}, C. Forsblom^{1,2}, L. Thorn^{1,2}, M. Saraheimo^{1,2}, D. Gordin^{1,2}, P.-H. Groop^{1,2}, FinnDiane Study Group;¹Folkhälsan Institute of Genetics, ²Department of Medicine, Division of Nephrology, Helsinki University Central Hospital, Finland.

Background and aims: Several single nucleotide polymorphisms (SNPs) have been identified for obesity in large genome-wide association studies on obesity in the general population. However, no such studies have been performed in patients with type 1 diabetes, a population also affected by obesity. Therefore, our aim was to study which SNPs affect the susceptibility to obesity in patients with type 1 diabetes using a genome-wide approach.

Materials and methods: Genotype data as well as data on BMI and covariates (age and sex) were available for 3211 adult Caucasian patients with type 1 diabetes. All patients participated in a nationwide multi-center study on diabetic complications. After quality control of the genotype data (Hardy-Weinberg equilibrium, minor allele frequency, genotyping rate, missingness and bad clustering), 509 938 SNPs remained. BMI was analysed with linear regression using PLINK v 1.07 and adjusted for age and sex. Results were corrected for multiple testing with Bonferroni correction.

Results: In this patient cohort, the prevalence of obesity, defined as BMI over 30 kg/m², was 10.3%, while 38.3% of the patients were overweight (BMI between 25 and 30 kg/m²). No SNP was significant ($P < 1.0 \times 10^{-7}$) after Bonferroni correction. However, five suggestive hits ($P < 1.0 \times 10^{-5}$) were observed on chromosomes 3p21, 14q31, 16p12, 18q21 and 20p11. The strongest association was in CST1 gene on chromosome 20 and increased BMI with 0.50 kg/m² per C allele ($P = 8.9 \times 10^{-7}$). Only one of the observed top hits was located in a gene previously associated with obesity (NRXN3 on chromosome 14). The other top hits were located in genes not previously associated with obesity; one exonic missense SNP (Trp > Arg) in CCDC71 (3p21) and intronic SNPs in XYLT1 (16p12), CTIF (18q21) and CST1 (20p11). However, although not among the top hits, the generally well replicated SNP in the first intron of FTO (rs8050136) was associated with BMI also in patients with type 1 diabetes and increased BMI with 0.31 kg/m² per A allele ($P = 6.1 \times 10^{-4}$).

Conclusion: In this preliminary analysis, we provide suggestive evidence for five new loci associated with obesity in type 1 diabetes. Only modest overlap was seen with results from genome-wide association studies in the healthy population suggesting partially different genetic causes for obesity in type 1 diabetes. Replication will be required to validate these loci.

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The transcriptional modulation involving mRNAs and microRNAs during development of T cells is associated with the emergence of type 1 diabetes mellitus in NOD miceT.A. Fornari¹, P.B. Donate¹, C. Macedo¹, E.T. Sakamoto-Hojo^{1,2}, E.A. Donadi³, G.A.S. Passos^{1,4};¹Genetics, University of São Paulo - Faculty of Medicine of Ribeirão Preto - FMRP/USP, ²Biology, University of São Paulo - Faculty of Philosophy, Sciences and Letters of Ribeirão Preto - FFCLRP/USP, ³Medical Clinic, University of São Paulo - Faculty of Medicine of Ribeirão Preto - FMRP/USP, ⁴Morphology, University of São Paulo - Faculty of Dentistry of Ribeirão Preto - FORP/USP, Ribeirão Preto, Brazil.

Background and aims: As early as one month of age, non-obese diabetic (NOD) mice feature pancreatic infiltration of autoreactive T lymphocytes, which destruct insulin-producing beta cells, leading to autoimmune diabetes mellitus (T1D) within eight months. Thus, we hypothesized that during the development of T1D, the transcriptional modulation of immune reactivity genes, as well as, modulation of microRNAs (miRNAs) may occur during thymocytes mature into peripheral CD3+ T lymphocytes. Our aim is to analyze the transcriptional modulation of mRNA and microRNAs during development of thymocytes into peripheral CD3+ T lymphocytes in the context of the emergence of T1D.

Materials and methods: Thymocytes and peripheral CD3+ T lymphocytes were obtained from pre diabetic and diabetic NOD mice, which were used to

prepare total RNA samples. The mRNA and miRNA fractions were labeled with Cy3 fluorochrome and hybridized with Agilent platform and the profilings of these cells were obtained through bioinformatics data analysis using GeneSpring software pipeline.

Results: The transcriptome of thymocytes and peripheral CD3+ T lymphocytes from pre diabetic and diabetic mice analyzed through microarray hybridizations identified 2771 differentially expressed genes. Hierarchical clustering grouped mice according to age/T1D onset and genes according to their transcription profiling. The transcriptional activity of thymocytes developing into peripheral CD3+ T lymphocytes revealed sequential participation of genes involved with CD4+/CD8+ T cell differentiation (Themis), tolerance induction by Tregs (Foxp3) and apoptosis (Fas) soon after T cell activation (IL4), while the emergence of T1D coincided with the expression of cytotoxicity (Crtam) and inflammatory response genes (Tlr) by peripheral T lymphocytes. Further analysis on post-transcriptional regulation of these genes showed involvement of the microRNAs miR-181a, miR-181c, miR-363 and let-7f, which were modulated in the course of T1D. Similarly to mRNAs the expression profiling of miRNAs were associated to the development of T1D.

Conclusion: These results are evidence for the association between the transcriptome (mRNA) and mirnome (miRNAs) of T lymphocytes and the manifestation of autoimmune diabetes mellitus.

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Association between interferon-induced helicase 1 (IFIH1) Ala946Thr polymorphism and the seasonal variation in the onset of type 1 diabetes

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Background and aims: The onset of type 1 diabetes is determined by an interplay between genetic and environmental factors. Among the environmental factors viral infections display a seasonal pattern contributing to the seasonal variation in the onset of type 1 diabetes. Previously we observed that the rs1990760 (A>G, Ala946Thr) polymorphism of the interferon-induced helicase 1 (IFIH1), a virus recognition receptor, confers a modest susceptibility to type 1 diabetes, namely the major A allele is associated with increased disease risk. The aim of the present study was to evaluate a possible association between the IFIH1 Ala946Thr polymorphism and the seasonal variation in the onset of type 1 diabetes.

Materials and methods: The IFIH1 Ala946Thr polymorphism was genotyped in 1056 patients of Eastern European ancestry with type 1 diabetes mellitus (age at diagnosis of diabetes: 8.6±5.1 years [range: 0.6 - 30.9 years], 539 males, 517 females). Type 1 diabetes onset was recorded in monthly intervals. Diabetes was classified based on clinical data; autoimmune markers were determined only if difficulties occurred in classification. Seasonality was characterized by cosinor test. The association between IFIH1 rs1990760 genotypes (AA, AG, GG) and trends in monthly manifestation of type 1 diabetes was evaluated by Mantel-Haenszel chi2 test. Ordinal logistic regression analysis was performed to study the effect of genotype and age on type 1 diabetes onset assessed by monthly intervals.

Results: Among the total cohort (n=1056), AA genotype was found in 436 patients (41.3%), AG genotype was observed in 484 patients (45.8%), and GG genotype was found in 136 patients (12.9%). Seasonal variation in manifestation of type 1 diabetes (highest rate in winter and lowest rate in summer period) was observed in the total cohort by the cosinor analysis. The protective GG genotype of IFIH1 polymorphism was less frequently found among the newly onset cases during summer versus winter period (9.29% vs. 12.88%, respectively). Conversely, the disease predisposing AA genotype was more frequently found among the newly onset cases in summer versus winter period (44.29% vs. 37.98%, respectively, p=0.0268). Significant effect of genotype (p=0.0266) but no effect of age (p=0.0888) was found on the seasonal variability of type 1 diabetes onset in the total cohort.

Conclusion: We confirmed the seasonal variation in the onset of type 1 diabetes in a large population of Eastern European ancestry. Importantly, we detected a significant seasonal variation in the predisposing effect of IFIH1 Ala946Thr polymorphism on type 1 diabetes susceptibility in our cohort, with a stronger prevalence of the protective GG genotype in the winter period, and lower prevalence in summer. On the other hand, the disease predisposing AA genotype was more frequent in the summer versus the winter periods. Thus, our findings suggest that this virus receptor gene may contribute to type 1 diabetes manifestation primarily in the summer period.

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Variants in IGF-I and IGFBP-1 and their effects on circulating IGF-I and IGFBP-1 levels in type 1 diabetes

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Background and aims: Recent genome-wide association study has shown the association of rs35767 (near IGF-I) with HOMA-IR and fasting insulin. IGF-I is an important regulator of fetal growth, type 2 diabetes and obesity. Linkage studies have shown IGF-I and binding protein polymorphisms to be also associated with T1DM. The protein products of these genes may influence antigen presentation and cellular immune response and thus have a role in T1DM pathogenesis. Genetic variations in the IGF-I and IGFBP-1 have shown to influence serum levels of IGF-I and IGFBP-1. Therefore, we aimed to study if the genetic variants of IGF-I (rs35767) and two SNPs in the IGFBP-1 gene (rs1065780 in the promoter region and rs4619 -Ile253Met) pose increased risk susceptibility to type 1 diabetes (T1DM) and influence serum concentrations of IGF-I and IGFBP-1 in subjects with T1DM and non-diabetic controls.

Materials and methods: A cohort of 250 T1DM subjects (mean age 23.11 ± 8.57 years) and 140 controls (24.77 ± 5.77) were recruited from Endocrine Clinic of Christian Medical College & Hospital, Vellore, India. Genotyping was performed using TaqMan allelic discrimination for rs35767 (IGF-I), rs2065780 and rs4619 (IGFBP-1). Fasting serum IGF-I was measured by RIA after separation of IGFs from IGFBPs by acid ethanol extraction and cryo-precipitation. IGFBP-1 were measured by in-house RIA (intra and inter-assay CV were 3% and 10%). Association of the IGF SNPs with serum IGF-I and IGFBP-1 were analysed using ANOVA. Logistic regression was used to investigate the association between the IGF SNPs in subjects with T1DM and controls.

Results: The minor allele frequencies of rs35767 (T), rs2065780 (G) and rs4619 (G) were 0.22, 0.23 and 0.20 respectively in T1DM subjects and 0.21, 0.21 and 0.18 among controls. The rs1065780 variant of IGFBP-1 was associated with increased risk of T1DM (OR 1.71, 95%CI 1.08-2.71, p=0.021). Serum IGF-I was lower in T1DM (Median 184µg/L Inter Quartile Range 137-242µg/L) compared to non-diabetic controls (325µg/L, 210-410µg/L), while serum IGFBP-1 was increased in T1DM (71.5µg/L, 34-141 µg/L) than controls (66µg/L, 44-93µg/L). Both were statistically significant (p<0.001). The risk allele (T) of rs35767 was associated with reduced serum IGF-I in controls (p=0.006). Paradoxically, the risk allele (G) of rs1065980 was associated with elevated serum IGF-I in controls (p=0.007).

Conclusions: The current study provides evidence for increased susceptibility to T1DM for rs 1065780 in the promoter region of IGFBP-1. Polymorphism in rs35767 and rs1065780 alter circulating levels of IGF-I among non-diabetic controls suggesting that variations in serum levels of IGF-I are genetically determined.

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Progression of type 1 diabetes associated autoimmunity and the high-risk HLA class II genotype

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Background and aims: The autoimmune process leading to beta cell destruction and type 1 diabetes is of variable duration and can be staged based on the number and quality of specific disease-associated autoantibodies. HLA polymorphisms are the main genetic risk factors for type 1 diabetes. The high risk DR3-DQ2/DR4-DQ8 (DR3/DR4) genotype mainly regulates the initiation phase of the disease process but does not seem to affect progression from multiple autoantibody positivity to clinical disease. However, there are some children who despite remaining positive for a single biochemically defined autoantibody develop diabetes, and information on the role of the high-risk class II HLA genotype in such subjects might help in understanding the process.

Materials and methods: The effect of the DR3/DR4 genotype on disease progression was compared between subjects with various numbers of autoantibodies in 249 prospectively observed autoantibody-positive children.

Results: The DR3/DR4 genotype was strongly associated with disease progression among those 54 children who never developed more than one

biochemical autoantibody ($p=0.003$, Kaplan-Meier log rank test), although the genotype was unrelated to disease development among those with more advanced autoimmunity. Subsequent analysis of ZnT8 antibodies revealed that this antibody was significantly associated with development of diabetes ($p=0.047$, Fisher's Exact test) but the DR3/DR4 genotype association with progression to clinical diabetes tended to remain after exclusion of these cases.

Conclusion: These results suggest that autoimmunity spreading associated with the high-risk HLA class II genotype is taking place in subjects positive for only one biochemical autoantibody although such spreading cannot be detected by current autoantibody tests.

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HLA-haplotypes, autoantibodies to beta cells: their role in the prediction of type 1 diabetes

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Background and aims: Risk of Type 1 Diabetes (T1D) development in mainly defined by susceptible haplotypes of HLA DR and DQ genes carriage and specific autoantibodies (Ab) presence. Prediction of early preclinical stage of T1DM.

Materials and methods: Predisposing and protective haplotypes (HLA-DRB1, genes DQ) together with immunological markers (ICA, GADA, IAA) have been studied in 143 discordant families, in normal siblings ($N=171$; mean age - 11.9 ± 5.8 years). Simultaneously HLA-genotyping was performed in 599 patients with Type 1 Diabetes (mean age - 7.5 ± 6.2 years). Control group included 200 individuals.

Results: During the trial the haplotypes susceptible to T1D [DRB1*4-DQA1*301-DQB1*302 (OR=4.7); DRB1*17-DQA1*501-DQB1*201 (OR=2.7); DRB1*4-DQA1*301-DQB1*304 (OR=4.0); DRB1*1-DQA1*101-DQB1*501 (OR=1.9); DRB1*16-DQA1*102-DQB1*502/4 (OR=2.4)] and protective [DRB1*15-DQA1*102-DQB1*602/8 (OR=0.08); DRB1*11-DQA1*501-DQB1*301 (OR=0.14); DRB1*13-DQA1*103-DQB1*602/8 (OR=0.16)] were defined in 599 T1D patients, belonging to Russian population. Analyzing of HLA haplotypes prevalence in patients' families and in control group it was revealed that susceptible haplotypes incidence was lower in normal siblings [DRB1*4-DQA1*301-DQB1*302, DRB1*17-DQA1*501-DQB1*201] - 45.6% and 31%, comparing with patients - 61.5% and 51.7%, respectively ($\chi^2=7.93$, $p<0.001$; $\chi^2=13.93$, $p<0.0001$); though it was higher comparing with control group - 8.5% and 10%, respectively ($\chi^2=66.7$, $p<0.0001$; $\chi^2=25.7$, $p<0.0001$). It should be noted that protective haplotypes [DRB1*15-DQA1*102-DQB1*602/8/DRB1*13-DQA1*103-DQB1*602/8] incidence was higher in normal siblings comparing with patients - 22.8% and 4.9%, respectively ($\chi^2=19.98$, $p<0.001$) and didn't differ from the incidence in control group - 22% ($\chi^2=0.03$, $p=0.85$). It is possible to suggest that protective haplotypes play their role in disease susceptibility lowering disease incidence among normal siblings. Three risk groups were selected in siblings of patients with T1D, considering that heterozygosity according to haplotypes [DQA1*0501-DQB1*0201 (DQ2) and DQA1*0301-DQB1*0302 (DQ8)] specifies the highest risk of T1D development. The high genetic risk group (DQ2/DQ8) included 13.5%, moderate risk group (DQ2/X, DQ8/X - 59.7% and low risk group (X/X) - 36.8%, where X is any haplotype, besides DQ2/DQ8. Incidence rate of two and three Ab types in high risk group was higher than in moderate and low risk groups (26.1%, 14.1%, 11.1% respectively; $p>0.05$; 8.7%, 2.4%, 0% respectively, $p<0.05$). In this case GAD was defined significantly more often in the group with DQ2/DQ8 genotype, comparing with groups of moderate and low genetic risk (65%; 37.9%; 18%, respectively; $p<0.05$). Ab to insulin were revealed in 19%; 12.9%; and 3.7% cases, respectively ($p<0.05$). T1D manifestation was observed in 11 individuals during 11 years of surveillance (6.4%). The disease was detected in 8.7% cases from high risk group, in 8.0% - from moderate risk group and in 3.3% - from low risk group. From those in whom disease developed high risk haplotypes (DQ2; DQ8) were revealed in 82% cases and only 18.2% from examined individuals didn't have susceptible haplotypes.

Conclusion: The data obtained confirmed once more once more, that DQ2, DQ8 haplotypes presence in normal siblings forecast high risk of T1D development, but even these haplotypes absence doesn't completely protect from disease development.

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Role of the repair enzyme PIMT in type 1 diabetes: studies in patients, dogs with diabetes and PIMT knock-out mice

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Background and aims: Post-translational protein modifications may create new antigenic epitopes and elicit autoimmunity. The enzyme PIMT, encoded by PCMT1, repairs isomerised Asn and Asp residues (IsoAsp). We showed that a compound up-regulating PCMT1, delays diabetes onset in the BB rat. The aim of this study was to assess the role of PIMT/PCMT1 in diabetes by studying several models.

Materials and methods: Patients (with GAD65 and/or IA2 antibodies) with a short duration (<5 years) of T1D were selected. As control groups, patients with T2D (<5 years since diagnosis and no complications) and healthy subjects matched for age and gender were selected. Samples from diabetic and non-diabetic dogs were collected. The expression of PCMT1 mRNA was assessed by qPCR. Transcription levels were normalized to β -actin by comparative Ct. Red blood cells were filtered and lysed. The cytosol was separated and depleted of haemoglobin and Western Blot for PIMT was performed. Heterozygous PIMT^{+/−} mice were crossed and their offspring, genotyped. PIMT knock-out mice were compared with their littermates at 5–6 weeks of age. Oral glucose (2g/Kg) and insulin tolerance tests (0.3 U/Kg) were performed. Differences were analysed using Mann-Whitney's U test.

Results: Blood samples are available from 82 participants, though only 52 (50% women) have been analysed. Median [range] PCMT1 expression tended to be reduced in the 19 T1D (3.03 [0.49–16]; $p=0.083$) as compared to the 28 controls (6.57 [0.81–32.67]), whereas no difference was found in the small group ($N=5$) with T2D (6.73 [0.42–15.56]). When only the 15 matched control-T1D pairs were analysed, differences were non-significant ($p=0.24$). PCMT1 expression was similar in the 8 diabetic (0.95 [0.31–3.89]) and the 9 non-diabetic (0.91 [0.88–1.09]) dogs ($p>0.2$). Western Blot analysis has so far been performed in 15 human subjects (5 per group), where it showed higher PIMT expression in controls than in T1D and T2D patients. The first litter of mice analysed included one knock-out, 5 heterozygous and 3 wildtype mice. Glucose averages 15 min. after the oral glucose load, were 351 mg/dl in the knock-out, 300 mg/dl in the heterozygous and 262 mg/dl in the wildtype subjects ($p>0.2$). The glucose nadir after insulin administration was 42, 39 and 40% of baseline, respectively ($p>0.2$).

Conclusion: PCMT1 mRNA expression was normal, both in patients with T1D and in dogs with diabetes. However, preliminary results point towards possible differences in protein expression, suggesting that it might be post-transcriptionally regulated. Furthermore, although sample sizes are still small, the Pcm1 gene seems to have a dose effect on glucose tolerance in mice. Ongoing experiments and sample collection should add to these results.

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Should I test this patient for maturity onset diabetes of the young?

A clinical prediction model to determine the probability of MODY

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Background and aims: Maturity-onset diabetes of the young (MODY) is often misdiagnosed as Type 1 diabetes (T1D) or Type 2 diabetes (T2D), resulting in inappropriate management. We aimed to produce a prediction model using simple clinical criteria that could be used in patients with young onset diabetes (diagnosed <35y) to assess their probability of MODY.

Materials and methods: Logistic regression was used to integrate clinical characteristics that could discriminate MODY (n=618) from T1D (n=284) and T2D (n=316). The β coefficients obtained were combined in regression equations to enable calculation of probabilities for MODY. Model performance was assessed by ROC curves and cross-validated misclassification rates.

Results: Characteristics predictive of MODY compared with T1D were lower HbA1c ($\beta=-0.54$) parent with diabetes ($\beta=3.17$), female sex ($\beta=1.32$), and older age at diagnosis ($\beta=0.1$). MODY was discriminated from T2D by lower BMI SDS ($\beta=1.01$), younger age at diagnosis ($\beta=-0.31$), female sex ($\beta=0.73$), lower HbA1c ($\beta=-0.46$), parent with diabetes ($\beta=1.68$), and not being treated with tablets or insulin ($\beta=-0.79$ and -1.16 , respectively). Both models showed excellent discrimination (ROC area under curve=0.94 and 0.98, respectively), and low rates of misclassification (9.5% and 5.6%). A 60% probability cutoff showed 93% sensitivity, 92% specificity for MODY v T2D, and 79% sensitivity, 92% specificity for MODY v T1D.

Conclusion: The clinical prediction models performed well in discriminating MODY from T1D and T2D and could be used to improve selection of patients for genetic testing. A web-based version of this model may be used by clinicians and patients, as well as the diagnostic laboratory.

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Cystatin C is not a good candidate biomarker for HNF1A MODY

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Background and aims: Cystatin C is an excellent marker of glomerular filtration rate (GFR). Several renal phenotypes have been described in HNF1A MODY, including tubulopathies. In addition, cystatin C expression is positively regulated by CRP the level of which is decreased in this form of monogenic diabetes. Thus, we hypothesized that serum cystatin C level might be altered in HNF1A MODY.

Materials and methods: We initially examined 51 HNF1A MODY patients, 56 subjects with type 1 diabetes mellitus (T1DM) and 39 with type 2 diabetes (T2DM), as well as 43 non-diabetic individuals (ND) from Poland. We used subjects from two UK cohorts (from Oxford and Exeter) as replication panels: 36 and 179 HNF1A MODY, 36 and 167 T2DM, respectively. In addition, 39 HNF4A, 174 GCK, 17 MODY5, 58 T1DM from Exeter were included. Cystatin C level was measured locally and all relevant clinical and biochemical variables collected. The data were analyzed with linear regression models, adjusting for sex age and estimated GFR (creatinine).

Results: In the Polish subjects, adjusted cystatin C level in HNF1A MODY was lower compared to T1DM, T2DM and ND ($p<0.05$ for all comparisons). In addition, in MODY, cystatin C-based GFR was 12.5 ml/min/1.73m² higher than the one calculated from serum creatinine level using the CKD-EPI formula (95% CI: 8.3 - 19.3, $p<0.0001$), while the two GFR estimates were similar

or cystatin C-based lowed in the other groups. However, these results were not confirmed in the UK cohorts. In the cohort from Oxford there were no significant differences in serum cystatin C levels between HNF1A MODY and T2DM ($p=0.9$). In the Exeter cohort cystatin C level did not differ significantly from any other diabetic group, except HNF1B MODY, where cystatin C-based GFR was the highest, and the difference reached borderline significance ($p=0.05$).

Conclusion: We were not able to confirm our hypothesis (supported by the initial results from the Polish study groups) that cystatin C level is altered by HNF1A mutations. Thus, this molecule cannot be considered a candidate biomarker for HNF1A MODY.

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Genetic testing of MODY: results from a Mediterranean population over 13 years

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Background and aims: MODY is a dominantly inherited form of diabetes that is usually diagnosed in young adults. A precise molecular diagnosis is important for prognosis and treatment, and provides important information to relatives. We aimed to describe the genetic tests requested at our hospital, one of the referral centres in Spain, for the last 13 years. Although we receive samples from all over Spain, our laboratory is the only testing centre in Catalonia. We also aimed to compare data in our region with others in Europe in which the diagnosis is largely optimized.

Materials and methods: 753 samples (607 probands and 146 relatives) were received for MODY genetic testing between October 1997 and December 2010. Minimal clinical information was available for these patients. Official Census figures (Catalonia, 2004) were used to calculate referral rates and minimum prevalence across provinces. Data were analysed using SPSS v 17.0.

Results: 510 (68%) samples were referred from adult clinics and 217 (29%) samples from paediatric centres. A mutation in glucokinase (GCK) or in hepatic nuclear factor genes (HNF1 α , HNF1 β and HNF4 α) were confirmed in 182/607 (31%) of probands. 765 genes were studied in probands (1.26 genes/patient). The frequency of mutations found was 27% in the gene sequenced firstly. 150 probands had 2 genes sequenced, of which 13% had a mutation. In 8 samples in which 3 genes were studied we did not identify mutations. GCK mutations were most frequent in both children and adults - 129 cases (70% of total; 93% and 52% in the paediatric and adult positive results, respectively). There were 47 HNF1 α mutations (25% of total and 39% of adults). Of 269 samples processed firstly for HNF1 α mutations, 14% were positive. In those cases with no detected mutation in HNF1 α , HNF4 α gene re-sequencing was performed in 14% of patients and GCK gene re-sequencing was performed in 17%. There only were 3 cases (1.6% of probands with a positive result) with HNF4 α mutations. Of the 21 samples sent to study of HNF1 β gene, 7 (4% of probands with a positive result) were positives. From 182 positive probands, we received 146 samples of relatives, of which 91 (62%) had a positive result, so we only detected an extra case in the family per 2 affected probands. 64% of samples were sent from Catalonian centres and 25 % from other Spanish hospitals. Data about referral rates and prevalence are shown in Table 1.

Conclusion: Our detection rate is similar than other countries. However, GCK mutation is the most frequent cause and we found a lower minimum prevalence. It could be due to a lower optimisation of screening of potential cases of HNF1 α mutation, a limited screening of HNF4 α and to inadequate study of relatives. The successive study of others, after a negative result in the first suspected gene, is in general only worth it for HNF4 α re-sequencing after an HNF1 α negative result.

	Population	Referrals	Referrals/ million	Pick-up rate (%)	Relatives with mutation	Cases/ million
Barcelona	5,117,885	331	65	29	47	28
Tarragona	674,144	25	37	36	4	19
Girona	636,198	20	31	25	0	8
Lleida	385,092	20	52	45	4	34
Catalonia	6,813,319	396	58	30	55	26

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Exome sequencing in monogenic diabetes

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Background and aims: The emerging high-throughput sequencing technologies enable much more extensive genetic analysis of single individuals than classical Sanger sequencing. Since monogenic diabetes is characterized by extensive genetic and clinical heterogeneity we therefore wanted to examine the performance of whole-exome sequencing for diagnosis of monogenic diabetes.

Materials and methods: We performed exome sequencing in patients thought to have monogenic form of diabetes and negative for mutations in GCK, HNF1A, HNF4A, HNF1B and INS. We excluded common, non-coding and synonymous variants using extensive filtering and bio-informatic analysis. Here we report our experiences from the initial analysis of the first nine probands sequenced, focusing on ultra-rare variants in a pre-defined set of 81 genes implicated in glucose metabolism.

Results: We obtained 44X average coverage of the entire targeted exome sequence and found 416 rare coding variants per individual. Among the nine individuals, 16 non-synonymous and nonsense mutations were found in our a priori list of 81 candidate genes. All but one variant were verified by Sanger sequencing. These candidate gene mutations included an HNF4A mutation (p.R89Q), despite previous negative Sanger sequencing; a PPARG mutation (p.R357X), which was unexpected given available clinical information at investigation; an ABCC8 mutation (p.A1366T) in an individual with adult onset of diabetes (which usually are not screened for ABCC8-mutations), and two additional mutations of possible etiological importance.

Conclusion: Exome sequencing led to a genetic diagnosis in at least 3 out of 9 patients who had previously undergone conventional re-sequencing of candidate genes with negative results. Thus, exome sequencing has the potential to provide more comprehensive molecular testing than Sanger sequencing in monogenic diabetes.

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Identification and functional characterisation of novel inactivating glucokinase mutations causing GCK-MODY in Slovakia

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Background and aims: Heterozygous inactivating glucokinase (GCK) mutations cause a subtype of maturity-onset diabetes of the young (GCK-MODY) characterised by mild stable fasting hyperglycaemia. Over 600 GCK mutations have been reported of which ~65% are missense. In many cases co-segregation in families has not been established and despite the importance of functional studies in ascribing pathogenicity for missense variants these have only been performed for <10% of mutations. The aim of the study was to sequence GCK in Slovakian subjects with a phenotype consistent with GCK-MODY and to explore the pathogenicity of identified variants through family and functional studies.

Materials and methods: GCK was sequenced in 128 probands. Co-segregation of identified variants was assessed where parental samples were available. Novel GCK mutations were kinetically characterised.

Results: Twenty-two mutations were identified in 36 families (including 17 missense) of which 5 (I110N, V200A, N204D, G258R, F419S) were novel. All mutations were absent from 200 normal chromosomes. Parental DNA was available for 23 probands (covering 14/22 mutations) and co-segregation established in all cases. Bioinformatic analysis predicted all missense mutations to be damaging. Eight (I110N, V200A, N204D, G258R, F419S, V244G, L315H, I436N) mutations were functionally evaluated. Basic kinetic analysis explained pathogenicity for 6 mutants which showed reduced glucokinase activity due to decreased rates of catalysis and/or affinity for glucose with relative activity indices (RAI) between 0.6–<0.001 compared to wild-type GCK (1.0). Mathematical modeling predicted thresholds for glucose stimulated insulin release (GSIR) between 5.9–7.0 mmol/L. For the remaining 2

mutants (L436N, L315H) additional molecular mechanisms were investigated including defects in regulation by glucokinase regulatory protein (GKRP) and protein stability. No differences in GCK inhibition by GKRP were observed for L315H however for I436N there was diminished inhibition compared to wild type enzyme (IC_{50} 20.3 ± 1.6mM vs. 13.8 ± 0.4mM respectively [$p < 0.02$]). Protein instability as assessed by thermal lability studies demonstrated that both L315H and I436N show marked thermal instability compared to wild-type GCK (RAI at 55°C 8.8±0.8% & 3.1±0.4% vs. 42.5±3.9% respectively [$p < 0.001$]). Mathematical modelling predicted GSIR thresholds of ~6.7mmol/L for both mutants.

Conclusion: We have identified 22 GCK mutations in 36 Slovakian families. For 25% of mutations selected for functional follow-up basic kinetic analysis was insufficient to demonstrate GCK inactivation and additional studies were required to demonstrate the molecular mechanism responsible for enzyme inactivation. Combining family, bioinformatic and functional studies can aid the interpretation of variants identified by molecular diagnostic screening.

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Three ancestral mutations are responsible for a third of glucokinase diabetes in the Czech registry

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Background and aims: The nation-wide Czech MODY registry records 135 independent families with glucokinase diabetes; of these, 21 carry the p.Glu40Lys mutation, 15 the p.315His, 13 the p.Gly318Arg and 10 p.Val33Ala. Moreover, one of these mutations is strongly geographically clustered within a single region of the country. Our aim was therefore to characterize their possible common origin, and estimate their age.

Materials and methods: Available members of the above mentioned families including the healthy relatives, as well as 94 unrelated non-diabetic subjects, were genotyped for 16 single nucleotide polymorphism markers covering a region of 14 million base pairs (Mbp) around the GCK gene (8 of these markers covering the innermost 1.5 Mbp). The assays were run in the TaqMan format assays on a 384-well LightCycler 480 platform. Haplotypes were inferred using the Haploview and Phase software, the evolutionary tree drawn using the MEGA5 program, and the age of mutations estimated using the DMLE+ program. Control data were utilized to re-check for the lack of linkage disequilibrium between the utilized markers in the general population.

Results: The p.Glu40Lys is carried on an 11-marker long conserved haplotype stretching over 9Mbp; the p.Leu315His is carried on a 7-marker conserved haplotype of 1.4 Mbp; the p.Gly318Arg is carried on a 8-marker haplotype of 4 Mbp. All these conserved haplotypes had an estimated frequency in the general population less than 0.5%. The p.Val33Ala mutation showed some indications of linkage disequilibrium to the markers, yet we could not define any significant haplotype. The mutations' age was estimated to be between 64 and 93 generations (95% confidence intervals 45–132). This indicates that the mutations may have arisen when the German tribes admixed with the Slavs.

Conclusion: Our data show that over a third of families recorded in the large Czech collection of glucokinase MODY carry one of ancestral mutations. This extent of dissemination of ancestral mutations has never been shown in other populations. Our data well demonstrate that glucokinase diabetes is unlikely to decrease the reproduction fitness, and we may even ask on the nature of a putative evolutionary advantage in times of famine and starvation, similar to the one postulated for the thrifty genotype in type 2 diabetes.

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Paternal mosaicism causing recurrent transmission of a novel insulin gene mutation associated with permanent neonatal diabetes

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Background and aims: We recently identified 2 siblings (one male and one female) from a Chinese family with diabetes mellitus diagnosed in the first 6 months of life. The parents and 2 other brothers do not have diabetes mellitus. We aim to perform genetic sequencing for mutation in the genes commonly

associated with neonatal diabetes mellitus (i.e. KCNJ11, ABCC8 and INS) and to investigate the possibility of somatic/germline mosaicism in the clinically unaffected parents to explain the recurrent transmission of permanent neonatal diabetes.

Materials and methods: Whole blood was collected from all members of this family and genomic DNA was extracted from peripheral leukocytes using standard procedures. Initial molecular genetic analysis for the affected daughter was performed at the molecular genetics laboratory in Exeter, United Kingdom. Subsequent analyses for the identified mutation were performed for the other family members at the genetics laboratory in Singapore General Hospital. Coding exon 3 of the insulin (INS) gene was amplified by PCR. Single-strand sequencing was carried out in both the forward and reverse directions using standard methods on an ABI 3100 (Applied Biosystems). Genomic DNA from the parents was also investigated for low-level mosaicism using an allele-specific quantitative real-time polymerase chain reaction (qPCR) assay for the identified mutation. Primers used for allele-specific amplification introduced a 2 nucleotide mismatch preventing amplification of the normal allele.

Results: We identified a novel heterozygous missense mutation in exon 3 of the insulin (INS) gene, c.326G>A, in both the affected siblings. This mutation resulted in the substitution of cysteine (C) residue by tyrosine (Y) at codon 109 of the human preproinsulin molecule, causing disruption of a critical disulphide bridge. In addition, DNA sequences of the father showed low level of the mutant allele. Quantification of the mutant allele in the lymphocyte DNA showed that he had approximately 40% of the mutant allele as his affected son, confirming somatic mosaicism.

Conclusion: We have identified the first 2 cases of permanent neonatal diabetes mellitus in Singapore due to a novel INS gene mutation, C109Y. Paternal mosaicism was responsible for the recurrent transmission of INS mutation in this family. To our knowledge, this is the second reported case of parental mosaicism for mutation of the INS gene.

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R325W SLC30A8 genotype is associated in a gender specific manner with post-transplantation diabetes mellitus

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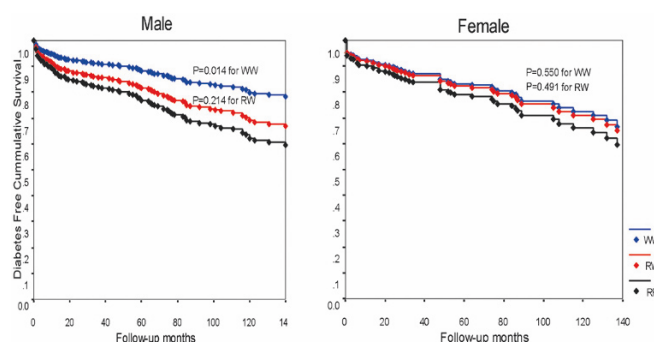
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Objective: The R325W nonsynonymous polymorphism (rs13266634) in the Zinc transporter-8 gene, SLC30A8, has been reported to be associated with posttransplantation diabetes mellitus (PTDM). Some studies reported that male gender is a risk factor of PTDM. We investigated the gender difference in PTDM development according to rs13266634 genotype.

Methods: A total of 624 unrelated renal allograft recipients (174 PTDM patients and 450 non-PTDM subjects) without previously diagnosed diabetes were enrolled. The genotyping of the SLC30A8 polymorphism was performed using real-time PCR.

Results: Among 624 patients enrolled, 403 were men and 221 were women. PTDM incidence was 28.04% (113/403) in men and 27.60% (61/221) in women. The prevalence of PTDM was 33.8% in patients carrying the R/R genotype, 26.8% in patients with the R/W genotype, and 19.8% in patients with the W/W genotype. The RW genotypes were associated with a tendency to increase PTDM risk [Hazard ratio (HR) = 1.28, P=0.214] and the RR genotypes showed increased PTDM risk [HR=2.13, P=0.014] in men. Whereas the effects of this genotype were not significant [HR=1.22, P=0.491 for R/W and HR=1.82, P=0.550 for WW] in women.

Conclusion: This study suggests that the SLC30A8 rs13266634 gene variation is associated with increased risk of development of PTDM in only men.



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Are population-based carriers of a mutation associated with cystic fibrosis patients more likely to have diabetes?

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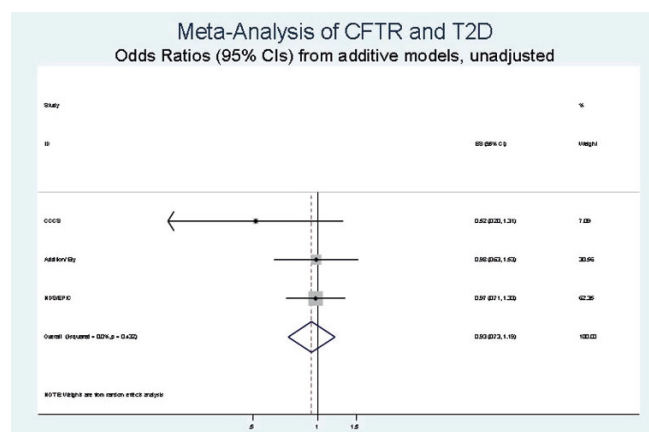
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Background and aims: Cystic fibrosis-related diabetes (CFRD) is increasingly common and occurs in some one third of adults with cystic fibrosis (CF), an autosomal recessive disorder. $\Delta F508$, a specific mutation within the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR), is the most common mutation causing CF and is carried by approximately 3-4% of individuals in the Northern European general population. Carriers of CFTR mutations, relative to non-carriers, may be more likely to develop medical conditions that accompany CF. Therefore, we tested whether the presence of $\Delta F508$ was associated with type 2 diabetes in the adult general population in the East of England.

Materials and methods: We genotyped the $\Delta F508$ variant in three type 2 diabetes case-control studies, including a total of 4,146 cases and 4,261 controls of European descent from the Norfolk Diabetes Case-Control Study (2,767 cases, 2,216 controls), the Cambridgeshire Case-Control Study (497 cases, 483 control), and the Addition/Ely studies (882 cases, 1,562 controls). Genotyping was performed at the MRC Epidemiology Unit Research Laboratory using a custom primer pair and custom probes (call rates 94%, 92%, 98%, respectively; Hardy Weinberg equilibrium p-values >0.4). We used logistic regression analyses to test the association between $\Delta F508$ heterozygosity and diabetes risk, adjusting for age, sex and body mass index (BMI). We used random effects meta-analysis methods to combine odds ratios across studies.

Results: 2.7% to 3.6% of controls were heterozygous for the $\Delta F508$ mutation. The association between $\Delta F508$ and diabetes risk was not significant in any of the individuals studies (figure 1), with a pooled odds ratio (95% confidence interval) of 0.93 (0.73; 1.19) and no heterogeneity between studies (I-squared 0%).

Conclusion: This study has identified no association between carrying the $\Delta F508$ mutation for CFTR and the presence of diabetes mellitus. If the presence of a single $\Delta F508$ inhibits insulin production, then this effect appears insufficient to cause diabetes. In summary, despite the high rates of diabetes observed in CF, carriers of the $\Delta F508$ mutation do not have a risk of diabetes different from that of non-carriers. Figure 1. Meta-analysis of the cystic fibrosis transmembrane conductance regulator (CFTR - $\Delta F508$) and type 2 diabetes



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Prevalence of diabetes and pre-diabetes in a cohort of Italian young adults affected by Williams Syndrome

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Background and aims: Williams Syndrome (WS) is a rare, multisystemic genomic disorder. While the natural history of the disease in childhood is quite well-known, few descriptions of the adult WS medical complications have been published. Recent data showed a high prevalence of diabetes in these patients.

Materials and methods: 22 young adult patients with WS (13 females, 9 males, age: 29.2±5.4 years) were studied. A 75g oral glucose tolerance test (OGTT) was performed in all but one who had known diabetes mellitus (DM). β -cell function was estimated with HOMA-B% and with insulinogenic index at 30 minutes ($\Delta I/\Delta G_{30}$). The insulin sensitivity was assessed with HOMA-IR, QUICKI and composite insulin sensitivity Matsuda index (ISI).

Results: Impaired glucose tolerance (IGT) was diagnosed in 12 subjects and DM in one, thus showing that 59% of this population had impaired glucose metabolism. ICA and GAD antibodies were absent. Logistic regression showed that the presence of impaired glucose metabolism was not associated with BMI ($P=0.267$, $B=0.118$), age ($P=0.977$, $B=0.004$), family history of diabetes ($P=0.653$, $B=0.747$), which are known risk factors for DM. WS subjects with IGT were more insulin-resistant than WS with normal glucose metabolism (HOMA-IR 2.3±0.9 vs 1.0±0.6, $P=0.008$; QUICKI 0.34±0.02 vs 0.39±0.04, $P=0.002$, ISI 3.2±1.4 vs 7.4±2.8, $P=0.006$). WS subjects with normal glucose tolerance compared to healthy adults matched for gender, age and BMI did not show any difference in β -cell function, insulin sensitivity, insulin levels during OGTT, but showed higher glucose levels at 120' after glucose load.

Conclusion: Given the high prevalence of impaired glucose regulation, adults with WS should be screened for diabetes or IGT. The reasons of this high prevalence of impaired glucose metabolism are not known. We excluded autoimmunity and usual risk factors for DM. However insulin resistance is likely to have a role in impaired glucose metabolism in WS subjects as it does in general population and lifestyle diabetogenic risk factors can have a role too. In fact these patients have limited physical activity due to mental deficiency or orthopaedic problems. However hemizygosity for a gene mapping to the WS chromosome region could be the factor responsible for the high frequency of diabetes and IGT in WS.

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Glycotoxin and autoantibodies are additive environmentally-determined predictors of type 1 diabetes: a twin and population study

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Background and aims: Type 1 diabetes mellitus is caused by destructive innate and adaptive immune responses. Serum diabetes-associated autoantibodies, to islet cell autoantibodies (ICA), glutamic acid decarboxylase (GADA), insulinoma-associated antigen-2 (IA-2A) and zinc transporter-8 (ZnT8A), reflect adaptive immunity, while carboxy-methyl-lysine (CML), an advanced glycation end-product, is associated with proinflammation. We assessed in a twin study whether serum CML levels and autoantibodies in type 1 diabetes were genetically determined, and whether they predicted diabetes in a population-based cohort.

Materials and methods: Serum CML and autoantibodies were determined prospectively in: a classical study of twins discordant for type 1 diabetes (32 monozygotic (MZ), 32 dizygotic (DZ) pairs); and, a population study of 7,287 normal subjects. In the latter, ICA positive subjects were followed for diabetes development. CML levels were determined by ELISA, autoantibodies by radio-immunoprecipitation and indirect immunofluorescence, HLA class II genotyping by sequence-specific oligonucleotides.

Results: CML levels were increased in diabetic and non-diabetic twins, population-based autoantibody positive and pre-diabetic subjects (all $p<0.001$). Twin CML correlations (r) were strong, irrespective of zygosity: model-fitting showed familial environmental factors explained 75% of variance. Autoantibodies were more frequent in diabetic than non-diabetic twins ($p<0.001$); but twin correlations were weak, irrespective of zygosity; non-shared environment explained all variance. Elevated CML in ICA positive subjects was a persistent and additive predictive marker of progression to diabetes.

Conclusion: Familial and non-shared environmental factors were strong determinants of serum CML and diabetes-associated autoantibodies, respectively. CML, a glycotoxin, is an additional diabetes-risk determinant with autoimmunity, and deserves consideration as a potential therapeutic target.

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Does the present incidence of type 1 diabetes in Poland in children fit the primary prediction for 2009?

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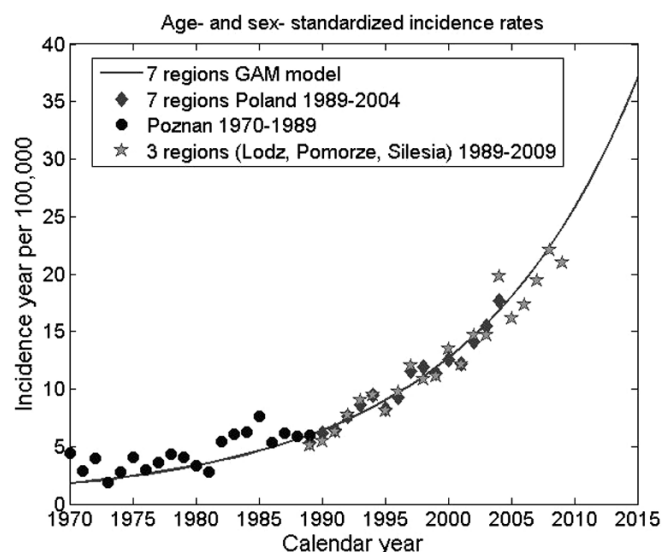
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Background and aims: Based on the rapid incidence increase of type 1 diabetes (T1DM) in Polish children (1989 - 2004) an incidence estimation for 2005 - 2025 was performed. The aim was to assess whether the temporal changes in incidence of T1DM in Polish children fit the primary predictive model.

Materials and methods: Children under 15yrs with newly diagnosed T1DM are ascertained prospectively (EURODIAB criteria) in several regional Polish registers. The prediction was constructed (GAM - general additive model) using incidence data collected 1989-2004 from 7 regions, covering ~35% population of Poland. The model was validated using Wielkopolska (Poznan) region data (1970-1989). The updated age- and sex- standardized incidence data (2005-2009) was derived from ~25% population (3 regions: Lodz, Pomorze, Silesia).

Results: During the last five years the age- and sex- standardized IR increased significantly from 16.1 [per 100,000] in 2005 to 21.0 in 2009 ($p=0.02$), which

is consistent with the GAM predicted dynamics (1.19 per year per 100,000) and it is higher than in the previous five-year period 2000–2004 (see figure). There were no significant differences between boys and girls. The most significant raise, consistent with the GAM prediction, was noticed among children aged 10–14 (from 16.0 per 100,000 to 23.4; annual increase 2.07 per year per 100,000 $p=0.02$). The significant increase of IR among children aged 0–4 and 5–9 was not observed (from 11.8 to 14.3; $p=0.24$ and from 20.1 to 24.4 per 100,000; $p=0.08$ respectively), which is disagreeing with GAM prediction. **Conclusion:** The present incidence of type 1 diabetes in Poland in children fits the primary prediction. The intuitively expected indicators of incidence saturation have not been observed yet. We confirm that the dramatic increase could cause real downstream effects on the national health care system in Poland.



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Pattern as well as pre-diabetic absence or presence of antibodies reveals phenotypic difference among LADA patients: results from the HUNT study

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Background and aims: Latent autoimmune Diabetes in the Adult (LADA) comprises a large part of all diabetes. Yet, the etiology of LADA is not completely clarified, nor distinctions from “classical” type 1 diabetes (T1D). Here we investigated cross sectionally the pattern, and prospectively also the pre-diabetic appearance of autoantibodies in LADA and adult onset T1D (age at onset >25) in a geographically defined and all-population-inclusive cohort of individuals >=20 years of age.

Methods: Patients with known diabetes were identified from the second (1995–1997) and third (2006–2008) population health survey in Nord-Trøndelag County, Norway (HUNT2 and HUNT3). Patients were classified by measurements of antibodies against glutamic acid decarboxylase (GADA) and by C-peptide. Patients classified as LADA were GADA positive and not treated with insulin within 12 months of diagnosis. Patients were classified as “classic” T1D if starting insulin treatment within 12 months of diagnosis and being either GADA positive, or GADA negative with fasting C-peptide levels <150 pmol/l. Measurements of antibodies against IA2 and ZnT8 were then carried out by means of RIA.

Results: Prevalent cases: Eighteen out of 162 LADA patients tested positive for other antibodies than GADA. Doubly and triply positive LADA patients vs. singly positive has had their diabetes diagnosis for a shorter time ($p=0.026$), had lower systolic blood pressure ($p=0.011$) and higher birth order ($p=0.020$), tended to have lower hip circumference ($p=0.057$) and higher non-fasting blood glucose ($p=0.058$). Age at onset was not associated with numbers of antibodies. Comparing LADA with adult onset T1D ($n=103$), LADA patients less frequently had more than one antibody (2 antibodies

$p=0.031$ and 3 antibodies $p=0.042$) but had higher GADA and IA2A-titers ($p=0.0002$ and $p=0.002$ respectively). Incident cases: Twenty-two out of 34 (65%) incident cases of LADA at HUNT3 were already antibody-positive 11 years earlier at HUNT2. Patients who were antibody positive at HUNT2 were diagnosed at younger age (54.4 ± 11.0 vs. 66.3 ± 8.63 , $p=0.002$), had lower blood pressure at HUNT2 ($p=0.048$) and were associated with higher GADA-titer at HUNT3 ($p=0.006$) than antibody negative patients. Thirteen out of 24 incident T1D cases (54%) were antibody positive already at HUNT2. There were no distinctive differences between antibody positive and antibody negative T1D patients either at pre-diabetes (HUNT2) or at overt diabetes (HUNT3). Antibody negative LADA at HUNT2 (pre-diabetes) were diagnosed at an older age than negative T1D ($p=0.004$).

Conclusion: Pattern, as well as pre-diabetic absence or presence of antibodies, reveals phenotypic differences among LADA patients. Differences were also found in comparison to adult-onset T1D. The differences observed could have therapeutic implications.

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Frequency of diabetic ketoacidosis in children with newly diagnosed type 1 diabetes in Serbia

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Background and aims: Diabetic ketoacidosis (DKA) is a frequent acute complication at onset of type 1 diabetes and is inversely related to incidence of type 1 diabetes. Compare to other European countries, incidence of type 1 diabetes in children aged 0–14 years in Serbia (13.1 per 100,000) indicates a moderate risk for this disease. Aim of this study was to determine the prevalence of DKA among children with newly diagnosed type 1 diabetes in Serbia in 2009.

Materials and methods: We conducted a cross-sectional study of newly diagnosed type 1 diabetes patients aged 0–14 years, from 10 regions in Serbia, identified through Serbian Diabetes Registry in year 2009. All comparisons were age and sex standardized to the national population. To compare the prevalence between the two groups, χ^2 -test was performed.

Results: DKA was observed in 39 (41.5%) of 94 newly diagnosed type 1 diabetes patients. There was no difference in the frequency of DKA between boys and girls (46.9 vs. 35.6%; $P=0.284$). The mean \pm SD age of the patients initially presenting with DKA was 8.2 ± 3.7 years. Boys were older than girls (9.6 vs. 6.3 years; $P=0.006$), and the proportion of boys increased by age. In agreement with previous studies, we observed that DKA was the most common among children aged <5 years (58.3%). Prevalence decreased significantly with age, and was the lowest in those aged 10 to 14 years (58.3% vs. 36.7%; $P=0.002$). Prevalence of DKA at onset of diabetes in summer was 33.3%, and in winter 46.2%. The difference was not statistically significant ($P=0.098$). The proportion of ketoacidosis increase concurrently with the proportion of newly diagnosed diabetes patients in winter.

Conclusion: The overall frequency of DKA in children with newly diagnosed type 1 diabetes in Serbia is high. Children less than 5 years of age have the highest risk of DKA at onset of diabetes. Increased public awareness about symptoms and signs of diabetes in childhood may reduce DKA rate at diabetes onset. In order to decrease frequency of diabetic ketoacidosis at diagnosis of type 1 diabetes there is an urgent need for prevention programmes to be designed.

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Pancreatic atrophy is apparent at the onset of type 1 diabetes in adults
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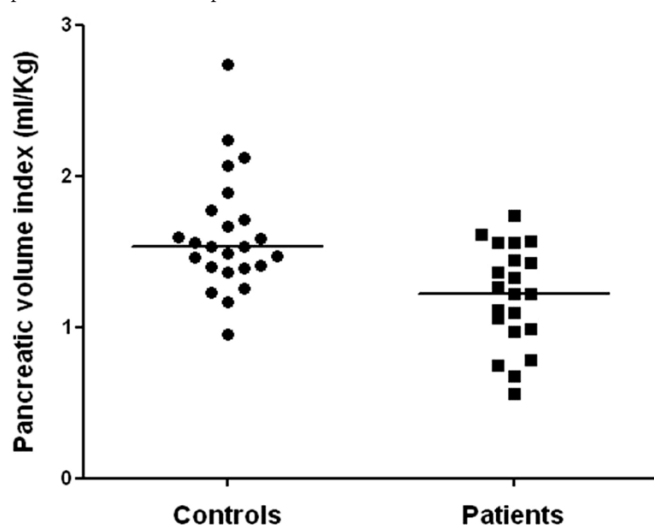
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Background and aims: Pancreatic atrophy is a characteristic finding in long-standing type 1 diabetes; pancreas size is reduced by 50% in adult patients who have had clinical disease for more than 10 years. Our aim was to determine whether pancreatic size was already reduced at disease onset and discover whether pancreatic volume in patients could be related to measures of metabolic and immune function.

Materials and methods: Pancreatic volume was measured in 21 male adult patients (median age 30 years, range 18 to 42 years) and 24 male healthy controls (median age 27 years, 19 to 44 years) by magnetic resonance imaging using a validated 3-D volumetric interpolated breathhold examination (VIBE) sequence; a T1 volume gradient echo sequence with fat saturation. Planar image slices throughout the pancreas were acquired at 2.5-mm intervals. Coded scans were analysed by an experienced radiologist who was ignorant of diabetes status. The patients had been diagnosed for a median of 3.6 months (range 1 to 7.9 months). Fasting blood samples were taken for measurement of glucose, C-peptide, HbA1c and the islet autoantibodies GADA, IA-2A and ZnT8A. Stimulated C-peptide was also measured in patient samples following injection of 1mg glucagon.

Results: Median pancreatic volume of the patients was 95 ml (range 37 to 141 ml) compared to 115 ml (range 67 to 195 ml) in the controls ($p=0.012$). Pancreatic volume correlated with body weight in patients and controls ($y=1.21x+15.7$, $p=0.012$). Following adjustment for body weight, median pancreatic volume index was found to be 20% less in patients (1.22 ml/Kg, range 0.56 to 1.74 ml/Kg) than in controls (1.53 ml/Kg, range 0.95 to 2.75 ml/Kg, $p=0.001$). No significant correlation was seen between pancreatic volume index in patients and diabetes duration, fasting glucose, HbA1c and stimulated C-peptide. Sixteen patients were positive for at least one islet antibody but there was no relationship between pancreatic volume index and the number of islet autoantibodies.

Conclusion: Pancreatic volume is reduced by 20% in patients with type 1 diabetes within months of diagnosis, suggesting that atrophy may begin years before the onset of clinical disease. Pancreatic atrophy within individuals is therefore a potential clinical marker of disease progression. The interaction between the exocrine and endocrine compartments within the pre-diabetic pancreas needs to be explained.



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Guangdong type 1 diabetes mellitus translational medicine study (1): clinical characteristics of 3,159 type 1 diabetic patients

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Background and aims: There are limited data on epidemiology and management of T1DM among Cantonese population. Given that, we initiated a series of province-wide studies to find out the current situation and to explore an appropriate management model for T1DM in Guangdong Province, China. First, we conducted this survey to provide the clinical characteristics of T1DM in Guangdong Province, China.

Materials and methods: A web-based registry database was established in August 2010. Data of established T1DM and newly diagnosed T1DM patients in 89 tertiary and secondary hospitals from all of the 21 cities in Guangdong Province were registered in the database from January 2000 to March 2011. Their medical records were collected and analyzed. The protocol and informed consent document were approved by the research ethics board of the 3rd affiliated hospital of Sun Yat-Sen University. All patients gave written informed consent.

Results: A total of 3,159 T1DM patients (1,698 males and 1,461 females) were enrolled in this study. The peak time of onset occurred at 20–29 years (mean age (SD) 28.8 (15.0) years) with about 26.93% of all patients, whereas the percentage of patients with the onset age <15 years was only 19.27%. The average disease duration was 5.7 (5.2) years. The mean BMI was 19.86 (3.45) kg/m² (data available in 1,886 patients). Family histories with diabetes in first-degree relatives were positive in 278 (8.80%) patients. Frequency of diabetic ketoacidosis (DKA) at onset of the disease was as high as 47.33% (data available in 2,846 patients). Among 1,428 patients with records on accompanying diseases, 42 (2.94%) had hypothyroidism, 51 (3.57%) had hyperthyroidism and another 5 (0.35%) patients had subclinical hyperthyroidism. Among patients with records on chronic diabetic complications, 17.48% (301/1,722) had diabetic nephropathy, 13.18% (221/1,677) had diabetic retinopathy, and 16.37% (281/1,717) had diabetic neuropathy. The morbidity of chronic complications increased with the duration of diabetes. For those with duration < 5 years, only 12.48 % (126/1,010) had nephropathy, while among patients with disease duration 5–15 and > 15 years, the percentage increased to 16.73% (94/562) and 54.74% (75/137), respectively. In terms of insulin regimens, 3.2% (101/3,159) were treated with insulin pumps. Among 1,376 patients with valid data of insulin categories, 49 (3.56%) were on animal insulin, 692 (50.29%) were on human insulin, and 635 (46.15%) were on human insulin analogues.

Conclusion: Our survey showed unique clinical characteristics of T1DM patients in Guangdong Province, China as compared with those in high or intermediate incidence regions: older age, higher morbidity of DKA at onset and lower BMI.

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Causes of death after initiation of dialysis among patients with childhood-onset type 1 diabetes in Japan: DERI mortality study

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Aim: To investigate causes of death after initiation of dialysis among patients with type 1 diabetes in Japan.

Methods: Subjects were 92 patients (37 males and 55 females) who died after initiation of dialysis, from 223 deceased cases taken from a total of 1,387 patients with childhood-onset type 1 diabetes. They were registered from two nationwide type 1 diabetes surveys in Japan and had been diagnosed with type 1 diabetes at less than 18 years of age between 1965 and 1979. All patients

were tracked for survival status until January 1, 2005 (follow-up period: 35 years), with status determined based on questionnaires sent to their attending physicians or the residents' records. Dialysis status and causes of death were identified through questionnaires or death certificates. The causes of death were divided into 9 groups (1. diabetic renal disease (RD); 2. acute diabetic complications; 3. accident/suicide; 4. cardiovascular disease (CVD); 5. infections; 6. malignant neoplasms; 7. other non-diabetic causes; 8. other diabetic causes; 9. unknown) and were also compared by follow-up period divided into quartiles (<14.8, 14.8–20.7, 20.8–26.0, >= 26.1 years). Statistical analyses were performed using SAS 9.1.

Results: The mean age at diagnosis of the 92 subjects was 11.1 ± 3.8 (SD) years, with a duration of diabetes of 23.9 ± 6.9 years. The mean follow-up period was 20.5 ± 7.6 years, and the mean age of death was 35.0 ± 7.3 years. The causes of death identified were RD: 35 cases, CVD: 25 cases, infections: 20 cases, other non-diabetic causes: 4 cases, unknown cause: 3 cases, acute diabetic complications: 2 cases, other diabetic causes: 2 cases, accidents and suicides: 1 case, and malignant neoplasms: 0 case. Among 25 patients who died from CVD, cerebral hemorrhage was the most frequent (10 cases, 40%), and myocardial infarction was the second most common cause of death (6 cases, 24%). The percentage of death from RD decreased as the follow-up period increased (<14.8 years: 56.5%, 14.8–20.7 years: 47.8%, 20.8–26.0 years: 34.8%, >=26.1 years: 13.0%). The percentage of death from CVD tended to increase as the follow-up period increased (<14.8 years: 21.7%, 14.8–20.7 years: 13.0%, 20.8–26.0 years: 26.1%, >=26.1 years: 47.8%). There was no trend identified in the percentage of death from infections according to the follow-up period (<14.8 years: 8.7%, 14.8–20.7 years: 34.8%, 20.8–26.0 years: 17.4%, >=26.1 years: 26.1%).

Conclusion: RD, CVD and infections were found to be the leading causes of death after initiation of dialysis among patients with childhood-onset type 1 diabetes in Japan. The percentage of death from RD was high in the earlier follow-up period although they had already initiated dialysis. Reasons for this might include the initiation of dialysis at a much later stage compared to recent patients with renal failure, because it was not until the early 1980s that dialysis became available for health insurance coverage in Japan, and that the technology for dialysis had not been as advanced. However, the percentage of death from RD decreased as the follow-up period increased while the percentage of death from CVD tended to increase as the follow-up period lengthened. Cerebral hemorrhage was the most frequent, and myocardial infarction the second most common cause of death among patients who died from CVD.

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PS 013 Which treatments are we using?

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Prevalence of antidiabetic medication use prior to and during pregnancy, 2001–2008

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Background and aims: With the increasing incidence of type 1 diabetes, the declining age at diagnosis for type 2 diabetes, and increasing prevalence of risk factors for gestational diabetes mellitus, more girls and women will be at elevated risk for DM or develop DM before or during their reproductive years. We examined trends in use of antidiabetic medications prior to and during pregnancy to explore how the use of drugs to treat diabetes is changing over time among pregnant women.

Materials and methods: We examined antidiabetic medication use in the 120 days before the last menstrual period (prepregnancy) and during the 2nd/3rd trimester of pregnancy among 135,111 women privately-insured and low income women (mean age 29.5 ± 6.0 years; 50% Hispanic, 26% White 12% Asian, 10% Black, 2% Other) with 161,217 births in a US managed care plan's hospitals from 2001–2008 using pharmacy records and infant birth certificates. Multivariate analysis was used to examine trends in antidiabetic medication use over the 8-year period after adjustment for repeated pregnancies, maternal age, race/ethnicity, and education using the SAS GENMOD procedure.

Results: Of the 161,217 births, 1.2% was to women using one or more antidiabetic drug prepregnancy while 4.5% was to women using antidiabetic medications during the 2nd/3rd trimester. Prior to pregnancy, 0.75% used metformin, 0.35% used insulin, and 0.23% used a sulfonylurea. Use of any antidiabetic medication pre-pregnancy \approx tripled from 2001 to 2008 (0.58% to 1.72%) with a 16% annual increase (95% CI 1.14–1.18) after adjusting for demographics and repeated pregnancies. Most of the increase was among women using metformin only (0.14% to 1.02%) versus other oral medications (0.20% to 0.26%) or insulin (0.24% to 0.40%). The use of antidiabetic medications increased by 7% per one year increase in maternal age (95% CI 1.06–1.08). During the 2nd/3rd trimester, 3.80% used insulin, 0.77% sulfonylurea, and 0.22% metformin. Use of antidiabetic medications increased by 17% from 2001 to 2008 (4.06% to 4.77%), with a 1.3% annual increase (95% CI 1.003–1.024) after adjusting for demographics and repeated pregnancies. The use of insulin declined (3.95% to 3.47%) while sulfonylureas (0.11% to 0.71%) and metformin (0.01% to 0.51%) use increased during pregnancy. Pregnant women who were older, had less than a high school education, and who were not non-Hispanic white were more likely to use these medications during pregnancy than their other pregnant counterparts (all p 's < 0.001). Overall, 0.4% births were to women who only used antidiabetic medications pre-pregnancy, 3.7% to women who only used them during pregnancy, 0.8% to women who used them both pre-pregnancy and during pregnancy, and 95.1% to women who did not use them. The majority of women who discontinued using antidiabetic medication after they become pregnant without starting another antidiabetic medication were using metformin; 60% of women using metformin pre-pregnancy did not use antidiabetic medications during pregnancy.

Conclusions: The increasing prevalence of antidiabetic medication use in the preconception period indicates a rise in DM and/or insulin resistance among childbearing women. However, the use of antidiabetic medications during pregnancy increased minimally over this same period in this diverse cohort. This study will continue to explore the association between risk factors and diabetes treatment on maternal and infant health outcomes.

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Sulphonylurea use in a woman with TNDM due to KCNJ11 mutation during pregnancy

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Background and aims: Children diagnosed with diabetes within the first 6 months of their life (referred to as neonatal diabetes) are likely to have monogenic diabetes. Approximately 25% of Transient Neonatal Diabetes Mellitus (TNDM) cases are caused by KCNJ11 and ABCC8 gene mutations. Sulphonylureas (SU) are effective in most KCNJ11 gene (encoding Kir6.2) related TNDM patients. There are few data on SU use in pregnancy in a KCNJ11 mutation carrier. We report SU use in a woman with TNDM due to the KCNJ11 mutation during pregnancy.

Materials and methods: A woman with the c.685G>A (p.E229K) Kir6.2 mutation became pregnant at the age of 16. The index patient in her family was her father. Her father and her brother have the same mutation and all of them were successfully transferred to sulphonylurea from insulin. The patient had been treated on 60 mg gliclazide daily for 4 years. She was not adherent to therapy, she used the tablets only every other day. Her HbA1c level was 8.2 % when she was referred to our clinic at the 11th week of her pregnancy. Despite of medical advice she made the decision to keep the baby by all means. She had no chronic diabetes complications. As her diabetes was very unstable during the years of insulin treatment and SU treatment had been optimal for years we decided to continue SU treatment. Her blood glucose was tested 3-4 times a day, Guardian Real Time Continuous Monitoring was performed in the second and in the third trimester.

Results: From the second day of directly observed gliclazide treatment she was normoglycemic during the whole pregnancy. This was supported by 2 CGMS, SMBG (3-4 times a day) and 3 consecutive HbA1c levels (5.8-5.2 -5.2%). There was no need to increase the dose of the SU. Cesarean delivery was carried out in the 38th week. The Apgar score of the newborn girl (weight 3010g) was 10 at 1 minute. The same mutation was identified from the umbilical cord blood. The baby has had no diabetes so far and her development is normal. Her blood glucose was checked regularly in the last 7 month. Our patient, the mother gained 10 kg during the pregnancy and her present BMI is 31kg/m². Since her labour she is not adherent to the therapy again, directly observed therapy is not possible. Her present SMBG range is 5-12 mmol/l, and the last 2 HbA1c was 9.1-9.3%.

Conclusion: The optimal dose of gliclazide was safe and effective for the patient and for the baby. In KCNJ11 gene caused mutation cases of TNDM the SU can maintain normoglycemia during pregnancy. This is the first case report of gliclazide use during pregnancy in TNDM caused by KCNJ11 mutation and the first identification of KCNJ11 mutation in a newborn. In some cases directly observed SU treatment is necessary in TNDM patients.

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Change of management status in newly diagnosed Korean type 2 diabetic patients: 3-year follow-up of Korean type 2 diabetes prospective cohort study

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Background and aims: Population based studies described that the prevalence of diabetic complication at time of diagnosis varies between 10% and 20%. About 50% of the subjects of UKPDS had substantial macro- or micro-vascular abnormalities at the time of T2DM diagnosis. The aims of this study were to describe the prevalence of diabetic complications among newly diagnosed diabetic patients who were enrolled to Korea national diabetes program (KNDP) constructing the type 2 diabetes cohort consisting of 12 university hospitals.

Materials and methods: Of 4,256 patients enrolled to the cohort, 712 newly diagnosed drug naïve patients were participated in this study. Metabolic profiles and diabetic complication status at diagnosis were investigated. And, data

of all 198 newly diagnosed type 2 diabetics who reached 3-year follow-up periods were compared with the baseline data including metabolic profiles and complication status.

Results: Mean age and HbA1c at the time of diagnosis were 51.3 ± 10.0 years and 8.2 ± 2.4% in 712 newly diagnosed drug naïve patients. The prevalence of metabolic syndrome at diagnosis was 88.8%. The prevalence of diabetic nephropathy, retinopathy, and neuropathy at the time of diagnosis were 21.4%, 8.4%, and 38.6% of 712 newly diagnosed drug naïve patients, respectively. During 3-year follow-up, lipid profile including total cholesterol, HDL-cholesterol, and LDL-cholesterol improved significantly compared with the baseline (P<0.001) in 198 subjects who reached 3-year follow-up. The glycemic control was improved in all glycemic parameter; fasting plasma glucose (FPG), postprandial plasma glucose (PPG), and HbA1c (FPG; 150.8 ± 54.6 vs. 128.9 ± 36.8 mg/dL, P<0.001, PPG; 266.7 ± 89.8 vs. 163.3 ± 65.4 mg/dL, P<0.001, and HbA1c; 8.0 ± 2.3 vs. 6.8 ± 0.9, P<0.001). Insulin secretory function measured by insulinogenic index was improved from 0.15 ± 0.16 to 0.38 ± 0.60 (P=0.013). At 3-year follow-up period, diabetic nephropathy assessed by urine albumin excretion was 20.8% of 198 subjects who reached 3-year follow-up, as compared with 25.5% at the baseline. Prevalence of diabetic retinopathy assessed by funduscopy was 15.2%, as compared with 9.8% at the baseline. Prevalence of diabetic polyneuropathy assessed by neuropathic symptom questionnaire was 17.7%, as compared with 34.1% at the baseline. There was significant improved carotid intima medial thickness after 3-year follow-up (P<0.001).

Conclusion: In conclusion, there had been an improvement in the management of glycemic control and cardiovascular risk factors while management status to prevent chronic complication was not enough. The introduction of intensive and systematic care into clinical practice should be necessary to prevent diabetes related chronic complication.

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Time to insulin initiation, glucose control and occurrence of diabetes related complications in France, Germany and UK from 2005 to 2010

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Background and aims: The market entrance of innovative oral anti-diabetics (OAD) like glitazones and DDP-4 inhibitors over the past years has enabled the time to insulin treatment for patients with diabetes mellitus type 2 (T2D). The study aimed to evaluate firstly whether the time to insulin has increased from 2005 to 2010 and to examine secondly the status of patients regarding diabetes-related events and glucose control before start of insulin therapy.

Material and methods: This retrospective cohort study analysed data collected by general practitioners in France, Germany and the UK using three longitudinal databases of the IMS® Disease Analyzer. The databases were searched for diabetes patients > 40 years (ICD-10 code E11; first diagnosis = index date) initiated on insulin therapy (ATC: A10C) between 1/2005 and 12/2010. Endpoints were (i) time from index date to initiation of insulin therapy (ii) the last HbA1c value within 6 months before start of insulin therapy (iii) percentage of patients with at least one macrovascular event (myocardial infarction, stroke, heart failure, coronary heart disease) between index date and first insulin prescription (iiii) mean number of different diabetes complications (myocardial infarction, stroke, heart failure, coronary heart disease, peripheral arterial occlusive disease, retinopathy, polyneuropathy, nephropathy) per patient from index date to first insulin prescription. Differences were tested by using Wilcoxon Rank Sum Test and Chi Square Test.

Results: Overall, 6,368 patients in Germany (mean age: 68.0 years [SD: 11.7], 51.7% male), 3,047 patients in France (mean age: 65.7 years [SD: 12.7], 53.7% male), and 1,998 patients in UK (mean age: 64.4 years [SD: 11.9], 55.3% male), had started with insulin therapy between 2005 and 2010. From 2005 to 2010, median duration until insulin initiation increased from 2.6 years to 4.2 years (p<0.001) in Germany, from 1.4 years to 3.2 years (p<0.001) in France and from 4.7 years to 5.6 years (p<0.001) in UK. In all three countries the percentage of patients with at least one macrovascular event before insulin initiation was higher in 2010 compared to 2005: in Germany 50.5% vs 45.3%, in France 26.3% vs. 18.5%, in UK 31.7% vs. 27.4%. In Germany, the difference was statistically significant (p<0.001). Mean number of different diabetes related complications per patient was slightly higher in 2010 compared to 2005: in Germany 1.31 (SD 1.29) vs. 1.17 (SD 1.20), in France 0.50 (SD 1.01) vs. 0.42 (SD 0.83), in UK 0.74 (SD 0.86) vs. 0.46 (SD 0.78). In UK, the difference was statistically significant (p<0.001). Median HbA1c values increased

from 7.80% in 2005 to 8.10% in 2010 in German patients ($p=0.0278$), from 8.55% to 8.60% ($p=0.0801$) in France and from 9.40% to 9.55% in the UK ($p=0.1344$).

Conclusion: This real-world data analysis show that time to insulin has significantly increased in Germany, France and UK from 2005 to 2010. HbA1c values of insulin naïve patients increased in all three countries. Concurrently, a clear trend indicating an increased morbidity regarding diabetes-related complications at insulin initiation can be observed in all three countries. Further research is needed to evaluate the underlying factors in Germany, France and UK more in detail.

Supported by: Sanofi-Aventis Diabetes Division

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Probability of the insulin initiation in type 2 diabetes patients depending on diabetes related complications

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Background and aims: Type 2 diabetic patients failing oral anti-diabetes medications need insulin. Not only inadequate glucose control but also other factors may have an influence on the duration until insulin therapy is initiated. The study aimed to evaluate whether chances for insulin initiation in patients with diabetes mellitus type 2 depend on diabetes-related events before start of insulin therapy.

Material and methods: This retrospective cohort study analysed data collected by general practitioners in France, Germany and the UK using three longitudinal databases of the IMS® Disease Analyzer. The analyzed database period was January 1995 to December 2010. The first diabetes diagnosis (ICD: E11) was defined as the index date. Age at index date was above 40 years. Main outcome measure was the initiation of insulin therapy depending on micro- and macrovascular complications recorded in the database after index date. A multivariate Cox regression model was fitted with the duration until insulin treatment initiation as dependent variable (up to 10 years after index date) and indicator variables for specific micro- or macrovascular diagnoses. Demographic and clinical data were also analyzed and included as confounders.

Results: Overall, 44,440 patients in Germany (mean age: 63.9 years [SD: 11.0], 51.2% male), 10,148 patients in France (mean age: 62.3 years [SD: 10.8], 58.8% male), and 25,499 patients in the UK (mean age: 63.4 years [SD: 11.4], 55.0% male), were diagnosed with type 2 diabetes in 1995–2009. Of them 9,747 (21.9%) in Germany, 702 (6.9%) in France and 3,936 (14.4%) in the UK began treatment with insulin within 10 years after index date. In all three countries patients with diabetes related complications in the time of oral treatment have significant higher chances to get the insulin therapy. In Germany, hazard ratios were 1.64 ($p<0.001$) for polyneuropathy, 1.45 ($p<0.001$) for cardiac insufficiency, 1.42 ($p<0.001$) for renal insufficiency, 1.41 ($p<0.001$) for retinopathy, 1.29 ($p<0.001$) for peripheral arterial disease, 1.21 ($p<0.001$) for stroke, 1.20 ($p<0.001$) for chd and 1.15 ($p=0.008$) for myocard infarction. In France, hazard ratios were 2.06 ($p=0.025$) for polyneuropathy, 2.54 ($p<0.001$) for cardiac insufficiency, 2.72 ($p=0.002$) for renal insufficiency, 3.23 ($p=0.103$) for retinopathy, 1.85 ($p=0.022$) for peripheral arterial disease, 1.54 ($p=0.003$) for chd. In the UK, hazard ratios were 1.54 ($p<0.001$) for polyneuropathy, 1.72 ($p<0.001$) for cardiac insufficiency, 1.20 ($p<0.001$) for renal insufficiency, 1.38 ($p<0.001$) for retinopathy, 1.61 ($p=0.086$) for peripheral arterial disease, 1.47 ($p<0.001$) for chd and 1.97 ($p<0.001$) for myocard infarction.

Conclusion: This real-world data analysis shows that patients affected from diabetes-related complications, are more frequently subject to a premature initiation of insulin therapy than patients having no complications. In this respect microvascular complications show higher impact than macrovascular complications. In routine care Insulin seems to be used to protect from recurrence or aggravation of complications.

Supported by: Sanofi-Aventis Diabetes Division

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Glycaemic control in 163 121 patients with type 2 diabetes on different glucose-lowering treatments: nationwide cross-sectional survey

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Background and aims: Current treatment guidelines advocate strict glycaemic control in type 2 diabetes. The clinical results, however, are likely to vary between treatments and groups of patients. The aim of this survey was to present HbA1c results among patients on the most common glucose-lowering regimens in clinical practice.

Materials and methods: Cross-sectional study in 163 121 patients reported to the Swedish National Diabetes Register (NDR). Patients on continuous pharmacological treatment were defined as patients who had been dispensed glucose-lowering agents at least two times, corresponding to six months use, during 2009. Patients using the 12 most common treatment regimens, each group representing >1% of all patients on glucose-lowering treatment, and patients on non-pharmacologic treatment were included. Clinical characteristics and results were compared using general linear modelling.

Results: Of the 108 618 patients treated with the most common pharmacological treatments, a total of 38,5% were treated with metformin, 6,4% sulfonylurea (SU), 15,5% metformin + SU, 3,4% metformin + meglitinide, 5,9% metformin + insulin NPH, 7,0% metformin + premixed insulin (direct-acting), 2,1% premixed insulin (direct-acting) + SU, 2,2% metformin + SU + insulin NPH, 3,2% metformin + direct acting insulin + insulin NPH, 4,7% direct acting insulin + insulin glargine, 6,3% premixed insulin (direct-acting). In addition, 54 503 patients underwent non-pharmacological treatment. There were statistically significant differences in mean (\pm SD) HbA1c between the groups, varying between $6.8\pm0.9\%$ in metformin (lowest) and $7.9\pm1.2\%$ in premixed with direct-acting insulin + SU (highest). The proportion of patients reaching HbA1c $\leq 7\%$ varied between 68,2% in metformin, and 24,5% in premixed with direct acting insulin + SU. The proportion of patients with HbA1c $\geq 9\%$ varied between 17.4% in premixed with direct acting + SU, and 2.3% in SU. In a subgroup of newly diagnosed patients (<3 years duration) mean HbA1c varied between $6.8\pm0.9\%$ in metformin monotherapy or in SU monotherapy, and $7.9\pm1.4\%$ in premixed with direct acting + SU. The patients with non-pharmacological treatment presented a mean HbA1c of $6.4\pm0.9\%$. The proportion of patients reaching HbA1c $\leq 7\%$ was 81,3% and 2,0% had HbA1c $\geq 9\%$. In a subgroup of newly diagnosed patients with non-pharmacological treatment mean HbA1c was $6.4\pm0.8\%$. Comparisons between genders showed higher proportions of men reaching HbA1c $\leq 7\%$, on all treatments; however, these differences were not statistically significant after adjustment for age.

Conclusion: There are profound differences in glycaemic control between different treatment groups, indicating a major challenge for diabetes care to optimize and develop treatments. Patients treated with metformin presented the best glycaemic control, apart from the non-pharmacologically treated. In most groups, including newly diagnosed patients or patients with non-pharmacological treatment, treatment targets are difficult to reach.

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Study of Once-Daily Levemir (SOLVE™) 1: clinical inertia in the initiation of insulin therapy in people with poorly controlled type 2 diabetes in real-life clinical practice

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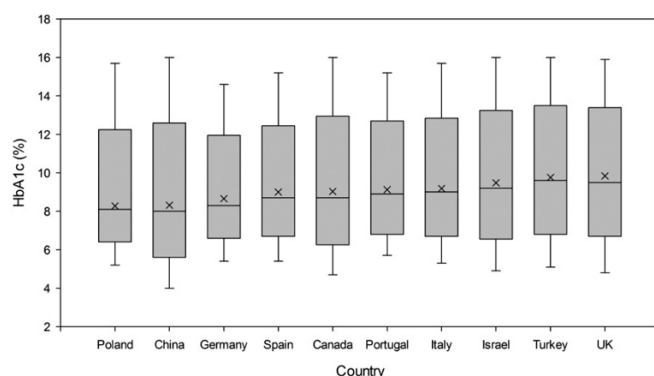
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Background and aims: Initiation of insulin therapy is considerably delayed, despite recently published guidelines from numerous countries encouraging the earlier and appropriate use of insulin and continued targeting of HbA1c values <7.0%. The aim of this analysis of SOLVE baseline data is to provide insights into the timing of insulin initiation in real-life clinical practice in relation to recommended glycaemic goals.

Materials and methods: SOLVE is a 24-week international observational study involving 10 countries evaluating the safety and effectiveness of once-daily insulin detemir in insulin naïve people with type 2 diabetes mellitus (T2DM) treated with one or more oral anti-diabetic drugs (OADs).

Results: A total of 14,785 participants have been enrolled in the study (mean age 61 ± 11 years, 53% male, and T2DM duration 10 ± 7 years). Prior to the initiation of once-daily insulin detemir, mean HbA1c was $9.0 \pm 1.7\%$, with 45% having HbA1c values $\geq 9.0\%$, and 25% having HbA1c values $\geq 10.0\%$. Only 7% of participants initiating insulin therapy had HbA1c levels of $<7.0\%$. Mean pre-insulin HbA1c (see figure) was highest in UK ($9.8 \pm 1.7\%$), Turkey ($9.8 \pm 1.8\%$), Israel ($9.5 \pm 1.6\%$), Italy ($9.2 \pm 1.5\%$) and Portugal ($9.1 \pm 1.7\%$); and lowest in Poland ($8.3 \pm 1.2\%$), China ($8.3 \pm 1.7\%$), Germany ($8.7 \pm 1.3\%$), Spain ($9.0 \pm 1.4\%$), and Canada ($9.0 \pm 1.7\%$). The proportion of patients with HbA1c $\geq 9.0\%$ ranged from 66% (UK) to 22% (Poland). The mean pre-insulin FBG of the total cohort was 10.6 ± 3.3 mmol/L; with substantial differences between countries ranging from 13.3 ± 4.2 mmol/L (Turkey) to 8.9 ± 2.0 mmol/L (Poland). Prior to insulin initiation, patients had received OAD therapy for mean 9 ± 7 years. Biguanides and sulphonylureas were the most commonly prescribed oral agents in all participating countries. Mean starting dose of insulin detemir for the total cohort was 0.16 ± 0.09 IU/kg, with a range of 0.12 IU/kg (Canada and UK) to 0.21 IU/kg (Turkey).

Conclusion: Despite documented benefits of timely blood glucose control and consensus guidelines encouraging earlier use of insulin replacement, there continues to exist considerable clinical inertia with respect to initiating appropriate insulin therapy, with nearly 50% of patients having HbA1c $\geq 9.0\%$. SOLVE highlights the importance of identifying and understanding regional and global practice habits and barriers to care, providing the basis for discussion and implementation of appropriate intensification of therapy in order to achieve glycaemic control earlier in the course of T2DM.



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Study of once-daily levemir (SOLVE™) 2: global baseline data and patterns of oral-antidiabetic drug (OAD) use before and after basal insulin initiation

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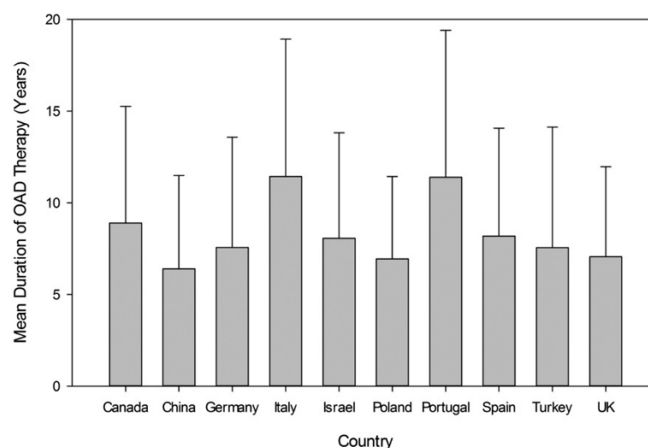
Background and aims: Considerable variations exist across countries in the approach to glycaemic management and therapy intensification for people with type 2 diabetes mellitus (T2DM). The aim of this cross-sectional baseline analysis is to provide insight into the timing of insulin initiation in real-life clinical practice in relation to glycaemic control and complexity of oral antidiabetic drug (OAD) use.

Materials and methods: SOLVE is a 24-week international observational study in 10 countries evaluating the safety and effectiveness of once-daily insulin detemir in insulin naive people with T2DM treated with one or more OADs.

Results: A total of 14,785 participants have been enrolled in the study: age 61 ± 11 years, 53% male, T2DM duration 9.9 ± 7.1 years, BMI 29.3 ± 5.3 . Pre-insulin HbA1c was $9.0 \pm 1.7\%$, with 45% of participants having HbA1c values $\geq 9.0\%$. Prior to insulin initiation, participants had received OAD therapy for 8.7 ± 6.7 years, with most patients on combination of 2xOAD (55%), 27% on monotherapy, and 17% on >2 xOAD. The most frequent OAD treatment regimen was a biguanide + sulphonylurea (38%). Overall, 82% were treated with a biguanide prior to initiating insulin therapy. Other OAD treatments included sulphonylureas (63%), glinides (16%), thiazolidinediones (TZD)

(13%), α -glucosidase inhibitors (13%) and DPP4 inhibitors (5%). Differences in OAD use were evident among participating countries. Pre-insulin duration of OAD use by country ranged from 11.4 ± 7.5 years (Italy) to 6.4 ± 5.1 years (China). DPP4 inhibitors were most commonly used in Portugal (46%), Israel (19%), Germany (19%), Spain (16%) and Canada (11%); whereas TZD were most often prescribed in Canada (26%), Turkey (23%) and the UK (21%). At the time of insulin initiation, the proportion of participants using glinides increased by 19%, whereas biguanides, sulphonylureas, TZD and DPP4 inhibitors were withdrawn in 3%, 18%, 32% and 37% of users, respectively. The proportion of participants on 2 and >2 xOAD agents also fell from 55% to 53% and from 17% to 11%, respectively.

Conclusion: There is substantial delay with respect to appropriate initiation of insulin replacement in real-life clinical practice, as reflected by the elevated baseline HbA1c in SOLVE. Considerable regional differences exist in the timing of insulin initiation and in the use of oral agents. Despite guideline recommendations to pursue target HbA1c $<7.0\%$, many patients have poor glycaemic control despite prolonged treatment with multiple oral agents. SOLVE provides an opportunity to better define and understand global and regional trends in diabetes treatment intensification and devise targeted treatment strategies to address glycaemic goal attainment in real world settings.



Clinical Trial Registration Number: NCT00825643

Supported by: Novo Nordisk A/S

PS 014 Genes, adiposity and type 2 diabetes

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Influence of type 2 associated polymorphisms on weight development in offspring of mothers with gestational diabetes

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Background and aims: Offspring of mothers with gestational diabetes (OGDM) are at increased risk to develop obesity and type 2 diabetes (T2D). Maternal pregravid obesity and large for gestational age (LGA) status of the child are predictors of obesity in OGDM. T2D risk alleles at the HHEX-IDE, CDKAL1 and PPAR γ 2 loci are associated with birth weight or weight development during childhood. The aim of this study was to determine whether these alleles are associated with fetal growth and growth during infancy in offspring of mothers with gestational diabetes.

Materials and methods: A total of 192 OGDM (76 female, median birth weight 3620g, 53 with LGA) who were enrolled in the prospective German GDM offspring study between 1989–2000 were studied. Data on birth weight (adjusted for gestational age and sex), BMI at the age of 2 years (expressed as age- and sex corrected standard deviation scores, SDS) and single-nucleotide polymorphism (SNP) genotyping of CDKAL1, HHEX-IDE, PPAR γ 2 and WFS1 were analysed. Statistical analysis was performed by linear regression.

Results: Birth weight percentiles were increased by 12.8 (95% CI:2.2–23.3, $p=0.02$) in OGDM with the homozygote T2D risk genotype (Pro/Pro) of the SNP rs1801282 on the PPAR γ gene region compared to OGDM with only one risk allele (none had no risk allele). BMI at the age of 2 years was associated with the PPAR γ rs1801282, WFS1 rs1801214 and HHEX-IDE rs10882102 gene loci. In the univariate analysis BMI-SDS was increased by 0.61 SDS (95% CI:0.2–1.1, $p=0.009$) in OGDM with two T2D risk alleles on the PPAR γ gene region compared to OGDM with one risk allele and by 0.31 SDS (95% CI:0.02–0.61, $p=0.039$) per T2D risk allele on the WFS1 gene region. In contrast, BMI-SDS was lower in OGDM with T2D risk alleles on the HHEX-IDE locus by -0.42 SDS (95% CI:0.7–0.1, $p=0.002$) per risk allele. This effect on BMI corresponded to a weight difference of 1.0 kg between offspring with two vs. no risk alleles ($p=0.02$). These effects remained significant after adjustment for LGA and maternal obesity in early pregnancy. Analysis of gene-gene interaction showed that the combination of two risk alleles from both, the PPAR γ and WFS1 gene region, is associated with the highest BMI-SDS at age 2 years (BMI-SDS 0.50 vs. -0.16 in offspring with 4 vs. 1 risk allele, $p=0.002$). However when adding the HHEX-IDE genotype to the model, the effect of the PPAR γ and WFS1 risk genotypes on BMI were no longer significant. In OGDM with 4 T2D risk alleles on the PPAR γ and WFS1 loci BMI-SDS was lower by 0.52 SDS per T2D risk allele on the HHEX-IDE locus (95% CI:1.18–0.27, $p=0.008$). The CDKAL1 polymorphism had no effect on weight development in OGDM. **Conclusion:** The recently reported association of the PPAR γ Pro/Pro genotype with increased BMI has been confirmed in our study on OGDM. The BMI increasing effect was highest in offspring with the simultaneous presence of WFS1 risk alleles. However, this effect is abolished by the BMI decreasing effect of HHEX-IDE risk alleles what is consistent with recent reports on the association of T2D susceptibility genotype at the HHEX-IDE locus with reduced BMI in offspring of parents with T1D.

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A gene expression signature for insulin resistance in hepatocytes caused by excess lipid

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Background and aims: Multiple pathways are implicated in insulin resistance, such as hyperlipidemia, elevated levels of pro-inflammatory cytokines and/or induction of oxidative or ER stress. The complexity of insulin resistance hinders effective characterisation and personalised treatment of type 2 diabetes. We propose that small sets of genes known as gene expression

signatures (GES) that reflect different insulin resistant states can be identified. The GES provides an unbiased transcriptional snapshot of the integrated cellular response to an insult.

Materials and methods: Hyperlipidemia-induced insulin resistance was modelled in FAO liver cells using palmitate (PA), and this phenotype was reversed using metformin and sodium salicylate. Global gene expression profiling was performed using microarrays. This was followed by bioinformatics analyses to identify the GES whose expression levels most significantly discriminated between the insulin resistant and insulin sensitive states. Insulin sensitivity was assessed by glucose production as the functional endpoint.

Results: In vehicle-treated FAO cells, insulin (0.1nM, 24h) decreased glucose production by 34 \pm 1% ($P < 0.0001$, $n=8$) indicating an insulin sensitive state. PA treatment (75 μ M, 48h) impaired the ability of insulin to decrease glucose production by ~80% ($P = 0.0035$, $n=8$) indicating a state of insulin resistance. The inhibitory effect of PA on glucose production was reversed by the addition of metformin (0.25mM) and sodium salicylate (2mM) in the final 24h of the PA treatment, returning glucose production to levels not significantly different to vehicle treated cells ($P = 0.078$, $n=8$). This study identified a set of five genes whose expression profile best defines the difference between insulin sensitivity and PA-induced insulin resistance in FAO liver cells. This complements our previous work identifying an inflammation based GES developed using TNF α to induce insulin resistance in 3T3-L1 adipocytes, and reversed by acetylsalicylic acid and troglitazone. We will next assess the ability of this GES to subgroup patients in human cohorts such as the San Antonio Family Heart Study.

Conclusion: This study has identified a set of genes whose expression profile best defines the difference between insulin sensitivity and PA-induced insulin resistance in FAO liver cells. The GES gives us the power to profile patients with type 2 diabetes, and then develop specific therapies targeted against each profile. The GES approach therefore opens the door for the development of personalised medicine for the treatment of type 2 diabetes.

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Long-chain polyunsaturated fatty acids modify the association between genetic variants in FADS and low density lipoprotein cholesterol

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Background and aim: Variants in the fatty acid desaturase (FADS) gene cluster have been associated with LDL, HDL and triglyceride concentrations in genome-wide association studies. FADS converts the α -linolenic acid (ω -3) and linolenic acid (ω -6) into long-chain polyunsaturated fatty acids. A high plasma concentration of long-chain ω -3 polyunsaturated fatty acids has been associated with decreased risk for metabolic syndrome and cardiovascular disease. We investigated the interaction between different intake levels of polyunsaturated fatty acids (PUFA) and the FADS polymorphism rs174547 (T/C) on LDL, HDL and triglyceride concentrations.

Materials and methods: We included 4,635 (45–68 years, 60% females) participants from the Malmö Diet and Cancer cardiovascular sub-cohort. Diet was assessed by combining a 168-item dietary questionnaire, 7-day menu book and a 1-h diet history interview. The percentages contributed by the specific PUFAs to total energy intake (E%) were divided in tertiles; low, medium and high. The General Linear Model was used to examine interactions, adjusted for several confounders.

Results: The minor C-allele (allele frequency 34%) of rs174547 was significantly associated with lower LDL ($p=0.03$). We observed a significant interaction between the FADS genotype and long-chain ω -3 PUFAs intakes on LDL plasma concentrations (p -interaction=0.01). The C-allele was significantly associated with lower LDL among individuals with low intakes of long-chain ω -3 PUFA (≤ 0.14 E%, $p<0.001$), but not among those with medium (0.14–0.28 E%) or high (>0.28 E%) intakes ($p=0.98$ and $p=0.86$, respectively). Significant interaction was observed between the FADS genotype and the ratio of α -linolenic and linolenic fatty acid intakes on the HDL cholesterol concentration (p -interaction=0.03). Across tertiles of the α -linolenic-linolenic fatty acid ratio there was no significant association between the C-allele and HDL concentration.

Conclusion: Our findings suggest that the dietary intakes of different PUFAs may modify the associated effect of the genetic variation in FADS on LDL and HDL concentration.

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The glucokinase regulatory protein rs1260326 polymorphism interacts with omega-3 PUFA to influence insulin resistance and inflammation in metabolic syndrome

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Background and aims: Glucokinase Regulatory Protein (GCKR) plays a central role regulating both hepatic triglyceride and glucose metabolism. Fatty acids are key metabolic regulators, which interact with genetic factors and influence glucose metabolism and other metabolic traits. Omega-3 polyunsaturated fatty acids (n-3 PUFA) have been of considerable interest, due to their potential to reduce metabolic syndrome (MetS) risk. We examined whether genetic variability at the GCKR gene locus was associated with the degree of insulin resistance, plasma concentrations of C-reactive protein (CRP) and n-3 PUFA in MetS subjects.

Materials and methods: Homeostasis model assessment of insulin resistance (HOMA-IR), HOMA-B, plasma concentrations of C-peptide, CRP, fatty acid composition and the GCKR rs1260326-P446L polymorphism, were determined in a cross-sectional analysis of 379 subjects with MetS participating in the LIPGENE dietary cohort.

Results: Among subjects with n-3 PUFA levels below the population median, carriers of the common C/C genotype had higher plasma concentrations of fasting insulin ($P=0.019$), C-peptide ($P=0.004$), HOMA-IR ($P=0.008$) and CRP ($P=0.032$) as compared with subjects carrying the minor T-allele (Leu446). In contrast, homozygous C/C carriers with n-3 PUFA levels above the median showed lower plasma concentrations of fasting insulin, peptide C, HOMA-IR and CRP, as compared with individuals with the T-allele.

Conclusion: We have demonstrated a significant interaction between the GCKR rs1260326-P446L polymorphism and plasma n-3 PUFA levels modulating insulin resistance and inflammatory markers in MetS subjects. Further studies are needed to confirm this gene-diet interaction in the general population and whether targeted dietary recommendations can prevent MetS in genetically susceptible individuals.

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Fat depot specific differences in mRNA expression of novel loci associated with waist-hip ratio

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Background and aims: Body fat distribution is one of the main predictors of obesity-associated complications. It might be hypothesised that changes in the gene expression or in gene function caused by genetic variants may

result in metabolic alterations related to obesity. We therefore investigated the adipose tissue expression profiles of 6 loci (TBX15, WARS, PIGC, STAB1, GRB14, ZNRF3), identified in a recent genome-wide association study (GWAS) for WHR, as their exact role in fat distribution remains largely unknown. Effects of genetic variants within these loci (rs984222 TBX15/WARS; rs6784615 STAB1; rs1011731 PIGC; rs4823006 ZNRF3; rs10195252 GRB14) on gene expression, WHR, and obesity related traits were examined.

Materials and methods: Paired samples of visceral (vis) and subcutaneous (sc) adipose tissue were obtained from 297 Caucasian men and women who underwent open abdominal surgery. Abdominal vis and sc fat area was calculated using CT scans and percentage body fat was measured by dual-energy X-ray absorptiometry. Gene expression was measured by quantitative real-time PCR by using TaqMan methodology. SNP genotyping was done using the TaqMan SNP Genotyping assay. Differences in mRNA expression between vis and sc adipose tissue were assessed using the paired t-test. Correlation analyses were carried out and multivariate linear relationships were assessed by generalized linear regression models. Two-sided P-values <0.05 were considered to provide nominal evidence for association (adj. age, gender).

Results: Differential expression between vis and sc adipose tissue could be observed for all tested genes. Except for STAB1 with higher mRNA expression in sc adipose tissue of males vs. females, there were no gender differences in gene expression in either tissue. Surprisingly, no correlation between WHR and fat mRNA expression of any of the genes was observed except for WARS whose expression in vis fat correlated positively with WHR in men. However, mRNA expression of several genes (e.g. WARS, PIGC, STAB1) correlated with BMI. Further, STAB1 expression in sc adipose tissue correlated with fat mass, and ZNRF3 expression in sc tissue correlated negatively with vis fat area. In addition, vis as well as sc expression of WARS and PIGC correlated negatively with both vis and sc fat area. Nominal associations were detected for rs984222 and TBX15 mRNA expression, rs4823006 and ZNRF3 mRNA expression, and rs10195252 and GRB14 mRNA expression, whereat rs984222 was also associated with BMI, sc and vis fat area as well as the GRB14 SNP with waist and waist-hip ratio in men (adj. age, BMI).

Conclusion: Besides rather moderate correlations of the expression levels with BMI or WHR, the most striking feature of expression of the 6 examined genes is the inter-depot variability as well as correlations with the vis and sc fat area. Our data further support the role of genes from recent GWAS in the regulation of fat distribution.

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The presence of methylation quantitative trait loci indicate a direct genetic influence on the level of methylation in adipose tissue

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Background and aims: Obesity is a condition of excess body fat that increases the risk for many diseases, including type 2 diabetes. Interestingly, studies have shown that upper body fat increases this risk, whereas lower body fat is related to reduced risk. It is of interest to understand if differences in disease risk are associated with expression and repression of certain genes and CpG methylation. As the measure of DNA methylation within tissue samples is quantitative in nature, methylation quantitative trait loci (meQTL) studies can be carried out. This project aimed to identify meQTL with the attempt find loci related to obesity as well as highlighting differences between these two types of fat depot.

Materials and methods: We analysed 294,025 genetic markers in 52 individuals and quantile-normalised differential methylation hybridization (DMH), which covers 51,430 methylation variable probes (40 for abdominal and 12 for gluteal adipose tissue) for association. A linear model was applied with an additive genetic effect and including the following covariates: gender, age, batch and case/control status for metabolic syndrome where appropriate. The meQTL analysis was limited to cis regions of 1 Megabase (MB). A false-discovery rate (FDR) threshold set to 5% was used to adjust for multiple testing.

Results: The distribution for the CpG methylation data of this study showed bimodality as found in previous studies, with a “hypomethylated mode” at 30% methylation and a “hypermethylated mode” at 70%. We did not detect any significant differences in methylation of CpG sites between the gluteal and the matching abdominal tissue samples. While we found no significant

meQTL hits in gluteal tissue (likely due to lower number of samples (N=12) in gluteal tissue), there were 701 in abdominal tissue after FDR correction ($p < 0.05$). These meQTL loci show a mean SNP-CpG distance of 182 Kilo-base (range 1 Base pairs - 1 MB). The top five meQTL hits were rs936266 ($p = 1.29 \times 10^{-15}$, closest gene MYH15), rs6456548 ($p = 4.34 \times 10^{-14}$, closest gene NRSN1), rs10233199 ($p = 4.34 \times 10^{-14}$, closest gene ZNF680), rs2293859 ($p = 2.05 \times 10^{-13}$, closest gene TDH) and rs3448 ($p = 4.45 \times 10^{-13}$, closest gene RHOA).

Conclusion: The presence of the meQTLs in abdominal adipose tissue indicates a direct genetic influence and consequently also an indirect influence on the general molecular phenotype of adipose tissue. Defining the genetic influence on both gene expression and CpG methylation in abdominal and gluteal adipose tissue can help towards characterizing these types of tissue and finding molecular pathways associated with obesity.

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Genome-wide gene expression in peripheral blood of type 2 diabetes patients and subjects with normal glucose tolerance: the population-based KORA Survey F4

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Background and aims: Genetics and lifestyle factors are both responsible for the manifestation of type 2 diabetes (T2D). Although genomics research has become more important over the last years, there are still wide gaps in our knowledge about the impact of genes on the development of T2D. A better understanding of the genetic effects may allow individualised prevention measures in the future. Therefore, we used peripheral blood to assess which genes are differentially expressed in T2D patients compared to subjects who had normal glucose tolerance (NGT).

Materials and methods: In the population-based KORA Survey F4 (Augsburg/Germany), whole-blood samples from fasting study participants were collected in PAXgene tubes between 8 and 11 a.m. After the isolation of total RNA, the whole-genome gene expression analysis was performed using the Illumina HumanHT-12 v3 Expression BeadChip. This BeadChip targets 48,804 transcripts, which represent more than 25,000 annotated genes. The transcripts were designed using the RefSeq and UniGene databases. For our analyses, we used RefSeq annotated 32,067 transcripts which are well-characterised. The association between gene expression and T2D were analysed by linear regression models using normalised gene expression levels as dependent variables. Multiple testing was corrected via multiple testing procedure based on Storey critical values which controls the false discovery rate (FDR) at a level of 0.05.

Results: This analysis is based on expression data from 515 individuals with NGT (47.2% men) and a BMI of 30.5 ± 3.7 kg/m² (mean \pm standard deviation [SD]) and 59 subjects with newly diagnosed T2D (54.2% men, BMI 27.8 ± 4.2 kg/m²). Subjects in both groups were aged between 62 and 81 years. In the age and sex-adjusted analysis, 311 transcripts were differentially expressed in T2D patients in comparison with subjects with NGT. After additional adjustment for BMI 9 transcripts remained significantly associated with T2D. The mean difference in gene expression for these 9 transcripts (8 up-regulated, 1 down-regulated in T2D patients) was $0.73 \times \text{SD}$ based on the SD calculated for the NGT subjects. Database searches indicated that the proteins encoded by these 9 transcripts are involved in the regulation of transcription and other pathways.

Conclusion: In our analysis, 311 transcripts were differentially regulated in peripheral blood of T2D patients in comparison with individuals with NGT. However, most of these associations were confounded by BMI. These findings will allow further analyses to characterise the involved genes in detail and to investigate their contribution to the manifestation of T2D.

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Reduced expression of cytochrome c oxidase and its copper chaperons leads to blunted glucose stimulated insulin secretion

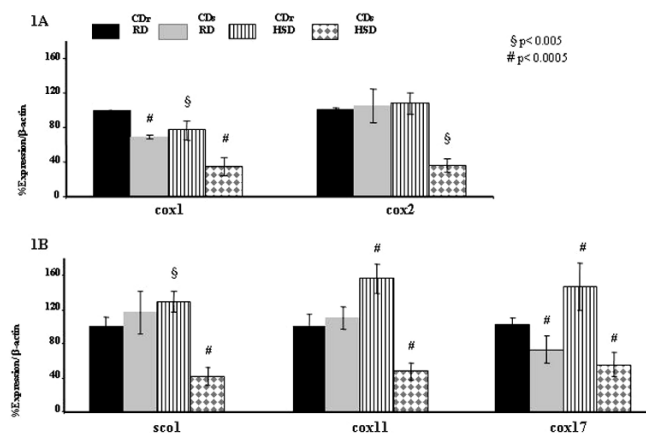
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Background and aims: Pancreatic β -cells couple mitochondrial oxidation of glucose to insulin secretion via cytochrome C oxidase (COX). Assembly and catalytic activity of COX depends on copper (Cu) ions distributed to COX by the Cu-chaperons Cox17, sco1/sco2 and cox11. Cohen diabetic sensitive (CDs) rats exhibit hyperglycemia due to reduced glucose stimulated insulin secretion (GSIS) when fed a high-sucrose-low-copper-diet (HSD) but remain normoglycemic on regular diet (RD). Resistant (CDr) rats remain normoglycemic on either diet. Feeding CDs rats with Cu-enriched HSD prevented β -cell dysfunction. We observed decreased protein levels of COX in CDsHSD islets in association with low COX activity. We examined the role of differential gene expression of COX and its Cu-chaperones in the regulation of GSIS.

Materials and method: Expression of COX subunit 1(cox1) and 2 (cox2) and of the Cu-chaperons Cox17, sco1/sco2 and cox11 was assessed in RNA extracted from the pancreases of CDs and CDr rats fed RD (CDsRD, CDrRD) or HSD (CDsHSD, CDrHSD) by qRT-PCR. **Results:** Expression of Cox1 and Cox17 were initially reduced in pancreases of normoglycemic-CDsRD rats compared CDrRD (figs 1A & 1B). Cox1 expression was further reduced in pancreases of hyperglycemic-CDsHSD rats [figure1A]. Expression of the Cu-chaperons increased in CDrHSD (sco1 +30%, cox11 +50%, cox17 +40%) and decreased in CDsHSD (sco1 -60%, cox11 -50%, cox17 -45%).

Conclusion: The CDs rats carry an initial inborn deficit in COX expression which is further augmented by lack of Cu. CDrHSD increased Cu-chaperones expression to compensate for low Cu concentration while CDsHSD lack this ability. Low expression of pancreatic Cu-chaperons in CDsHSD pancreases could lead to lower Cu concentration in the mitochondria minimizing Cu ions transfer to COX, thereby diminishing Cox 1 & Cox2 expression, protein levels and activity leading to diminished GSIS



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Genetic variation at the STAMP2 locus interacts with promoter variants of TNF α and IL-6 pro-inflammatory cytokines on the risk of type 2 diabetes and the metabolic syndrome

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Background and aims: STAMP2 (six transmembrane protein of prostate 2), initially discovered in the prostate, is mainly expressed in adipose tissue. In mice, STAMP2 is up-regulated by TNF α and IL6 in adipocytes. Conversely, STAMP2 expression seems to down regulate the pro-inflammatory cytokine expression and/or the insulin resistant response to them. In the D.E.S.I.R. prospective study, the IL6 promoter polymorphism (SNP) -174G/C was associated with type 2 diabetes (T2D) but not with the metabolic syndrome (MetS), and no association with T2D or MetS was found with TNF α -308G/A and STAMP2 SNPs. We now assessed the interactions between STAMP2 SNPs, and TNF α and IL6 promoter variants, on the risk of T2D and the MetS in the D.E.S.I.R. cohort.

Materials and methods: Over 4000 men and women could be analyzed for the prevalence and the 9 year incidence of T2D and the MetS. The interactions between SNPs were tested by logistic regression. The odds ratios to develop T2D and the MetS were assessed separately, by logistic regression, adjusting for age, sex, and BMI. Nine SNPs of STAMP2, the TNF α -308 G/A and the IL6 -174 G/C were investigated.

Results: The STAMP2 rs12386756 G>A and IL6 -174 G/C interacted for the susceptibility to T2D (p interaction =0.03 and 0.05 for prevalence and incidence respectively). The IL6 -174 G/C was significantly associated with T2D only for carriers of the A allele (for prevalence: OR, 0.67; 95%CI, 0.49-0.91; for incidence: OR, 0.59; 95%CI 0.40-0.88). The STAMP2 rs8122 C/T SNP interacted with TNF α -308 G/A for susceptibility to MetS (p=0.04 and 0.01 for prevalence and incidence respectively). The -308 A allele was associated with MetS in CC genotype only (for prevalence: OR, 0.70; 95%CI 0.53-0.93; for incidence: OR, 0.61 95%CI, 0.43-0.86). Weaker interactions between TNF α -308G/A and STAMP2 rs1026311 G/C and rs1981529 T/C were found.

Conclusion: The allelic variation at the STAMP2 locus modulates the effects of the promoter variants of IL6 and TNF α genes on T2D and the MetS in a prospective cohort. This could be indicative in humans of a cross talk between STAMP2 and pro-inflammatory cytokines, such as that found in mice.

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Genome-wide sequencing and gene expression QTL mapping in the Goto-Kakizaki rat identify Ascl3 as a regulator of adipocyte function

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Background and aims: Genetic studies in humans and animal models have identified numerous diabetes and obesity susceptibility loci that remain to be functionally characterized. We aim to identify candidate genes accounting for a quantitative trait locus (QTL) linked to adiposity and glucose tolerance mapped to chromosome 1q33 in a F2 cross and in congenic strains derived from the Goto-Kakizaki (GK) rat model of spontaneous diabetes mellitus and Brown Norway (BN) controls.

Materials and methods: Illumina gene expression bead chips were used to quantify the expression of 20,000 transcripts in adipose tissue from F2 (GKxBN) hybrids (n=138) and a congenic strain containing GK alleles at the adiposity/glucose tolerance QTL introgressed onto a BN genetic background. Gene expression QTLs (eQTLs) were mapped in the F2 cross using the R statistical package and validated in the congenics by qRT-PCR. We used next-generation sequencing technologies to sequence the GK rat genome and identify genetic variants in QTLs. Fibroblasts were isolated from adipose tissue of BN, GK and congenic rats to measure cell proliferation (Hoechst, ³H incorporation and Brdu) and differentiation (Oil-Red-O). The effect of promoter variants in candidate genes was assayed by luciferase assay.

Results: We identified 585 statistically significant eQTLs (LOD>9). Among the 172 positional candidate genes mapped to 1q33, 44 correspond to eQTLs

and 27 showed evidence of differential expression between congenics and BN controls. Following analysis of polymorphisms in the GK genome sequence, we were able to identify false positive eQTLs and to carry out functional analysis of polymorphisms in positional candidate genes. We selected Ascl3 (eQTL LOD=43 in the F2) which was found strongly down regulated in the congenics (-84%; p=6.10⁻⁴) when compared to BN. We identified an 8bp deletion in Ascl3 promoter in the GK, which causes a 38% decrease in transcriptional activity. Furthermore, we found a 70% significant decrease in cell proliferation and a 22% decrease in cell differentiation (p=0.02) in congenic and GK strains when compared to BN.

Conclusion: Combined analysis of genome sequence and gene expression data in an experimental F2 cross and in congenic strains allowed the identification of Ascl3 as a transcription regulator accounting for a QTL linked to adiposity and glucose tolerance in the GK rat. These results provide new insights into the transcriptional regulation of adipocyte function.

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Sorting nexin 19 regulates the number of dense core vesicles in pancreatic beta cells

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Background and aims: The sorting nexins (SNX) belong to a large family that is involved in protein sorting and intracellular trafficking. SNX19 is a 992 long amino acid member of this family which has a phox (PX) domain (a binding motif to phosphatidylinositol) at the COOH-terminus and a PX-associated (PXA) domain at the NH2-terminus. The function of SNX19 is not known but it binds to the dense core vesicle (DCV) transmembrane protein IA-2. IA-2 is a major autoantigen in type 1 diabetes. This molecule is present in neuroendocrine cells throughout the body and the knockout of IA-2 in mice results in the impairment of the secretion of hormones and neurotransmitters resulting in a variety of phenotypes characterized by impaired insulin secretion, glucose intolerance, female infertility, abnormalities in learning and behavior and loss of circadian rhythm. Overexpression of IA-2 in MIN6 cells and a rat pheochromocytoma cell line PC12 cells increased insulin secretion and dopamine release, respectively. Because SNX19 binds to IA-2, the present experiments were initiated to study the effect of the knockdown and reconstitution of SNX19 in MIN6 cells on the biology and physiology of DCV including the half-life and number of DCV and the cellular content and secretion of insulin.

Materials and methods: Stable SNX19 knockdown (SNX19KD) MIN6, a mouse pancreatic beta-cell line, and stable SNX19-reintroduced SNX19KD MIN6 were established. Quantification of DCV, lysosomes and autophagy were performed by electron micrographs. The half-life of DCV was detected by pulse-chase experiment. Insulin content and insulin secretion were measured by ELISA. Lysosomal activity was determined by cathepsin D activity kit.

Results: Insulin secretion and content were decreased in stable SNX19KD MIN6 cells compared to those in control MIN6 cells. Electron micrographs showed that the number of DCV in SNX19KD MIN6 cells was decreased about 75% and that the size of DCV was about 40% less, respectively, compared to those in control cells. Moreover, when SNX19 was reintroduced in SNX19KD MIN6 cells, insulin content, insulin secretion, and the number of DCV were increased. The half-life of DCV was decreased in SNX19KD cells but was prolonged in SNX19KD cells in which SNX19 was reintroduced. The number of lysosomes, activity of lysosome enzyme cathepsin D and LC3-II, an autophagic marker, were increased about 3-fold in SNX19KD MIN6 cells compared to those in control cells. On the other hand, they were decreased to about half to one third in SNX19-reintroduced SNX19KD MIN6 cells.

Conclusion: SNX19 regulates the number of DCV and insulin content by stabilizing DCV in beta-cells.

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Involvement of heparan sulphate 3-O-sulfotransferase isoform-1 for insulin secretion pathway

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Background and aims: Heparan sulfate (HS) is linear polysaccharides consisting of repeating disaccharide unit backbone onto which is superimposed a complex pattern of modifications, most notably addition of sulfate groups. The produced polymorphic sulfated sequence motifs are responsible for binding to a variety of signaling molecules and modulating their biological functions. Recently, we found HS is localized exclusively around beta cells in islets of adult mice and required for islet morphogenesis, beta cell proliferation and insulin secretion. Depletion of HS in beta cells using the technique of knockout mouse or enzymatic removal of HS decreased glucose-induced insulin secretion (GIIS). However the contribution of sulfate fine structure in HS to β -cell function is still unclear. The aim of this study is to clarify how sulfate group(s) in HS surrounding beta cells affects insulin secretion and cell growth.

Materials and methods: Subclone of MIN6, insulin-secreting mouse insulinoma cell line, was isolated based on effective GIIS and high expression of

HS. GIIS was examined in islets, MIN6 and isolated subclone MIN6T3 after removal of sulfate groups by sodium chlorate (a competitive inhibitor of glycosaminoglycan sulfation). Quantitative RT-PCR was used for analyzing mRNA expression of HS modification enzymes and components of signaling pathway. Expression of 3-O-sulfotransferase isoform-1 was silenced and GIIS was examined.

Results: GIIS in the sodium chlorate-treated mouse pancreatic islets, MIN6 and its subclone MIN6T3 showed about 62.4%, 52.4% and 62.5% decrease compared to the controls ($p < 0.01$). Addition of exogenous soluble HS in culture recovered GIIS of the MIN6 and MIN6T3 treated with sodium chlorate. The HS modification enzymes, including N-deacetylase/N-sulfotransferases, C5-epimerase and 6-, 3-O-sulfotransferases were detected in MIN6T3. Among these genes, mRNAs of several N-, O-sulfotransferase enzymes were induced by sodium chlorate-treatment. Silencing of a 3-O-sulfotransferase isoform-1, one of these upregulated enzymes, by RNA interference reduced GIIS to less than 70% of control treatment ($p < 0.01$). Insulin secretion induced by KCl was, however, unaffected in MIN6T3 with RNA interference. To determine the expression pattern of the components of signaling pathway downstream of HS especially affected by 3-O-sulfate modification, we used quantitative RT-PCR to evaluate the expression of genes encoding of Fgf, Notch, Hedgehog, Wnt and Tgf β . In 3-O-sulfotransferase isoform-1 silenced MIN6T3, the mRNA levels of Jagged2 and Delta-like4, the Notch ligands, were increased when compared to the non-treatment control (1.2- and 3.3-fold, respectively).

Conclusion: Our data indicate that 3-O-sulfate groups modified by 3-O-sulfotransferase isoform-1 plays important role(s) in the insulin secretory pathway, selectively through upstream of membrane depolarization in beta cells. Our result also suggests Notch signaling pathway including Jagged2 and Delta-like4 may be involved in the signaling pathway in beta cells downstream of HS.

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Attenuation of glucose-induced insulin secretion from pancreatic beta cells by intermittent hypoxia via down-regulation of CD38

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Background and aims: Sleep apnea syndrome (SAS) is characterized by recurrent episodes of oxygen desaturation during sleep, the development of daytime sleepiness, and deterioration in the quality of life. Up to 30% of the adult population in Western countries are thought to be affected by asymptomatic SAS and approximately 2-4% by symptomatic SAS. Accumulating evidence suggests the association of intermittent hypoxia (IH), a hallmark of SAS, and type2 diabetes independently on body mass index and waist circumference. In addition to the development of glucose intolerance and insulin resistance, the progression to type 2 diabetes is dependent on the impairment of glucose-induced insulin secretion (GIS) from pancreatic beta cells. However, the direct effects of IH on GIS are elusive.

Materials and methods: Hamster insulinoma HIT-T15 cells and isolated rat islets were exposed either to 64 cycles/24 h of IH (5 min hypoxia (1% O₂)/10 min normoxia (21% O₂)), or normoxia for 24 h. After the treatment, the medium was discarded, and islets and HIT-T15 cells were incubated either in RPMI1640 medium containing 5.5 mM or 22 mM glucose in normoxia for 30-60 min. After glucose stimulation for 30 min (60 min in HIT-T15), the medium was recovered. Insulin concentration in the islet medium was determined by a rat insulin ELISA kit. Real-time RT-PCR of insulin, CD38, glucose transporter 2 (Glut2), glucokinase (GK), sulfonylurea receptor1 (SUR1), and L-type Ca channel1.2 (Cav1.2) was performed using normoxia- or IH-treated islet RNA as template. To evaluate the effect of IH on CD38 promoter activity, HIT-T15 cells were transiently transfected with the reporter plasmid consisting of a luciferase reporter gene under the control of human CD38 promoter, and luciferase activities were measured after the exposure to IH or normoxia. GIS from HIT-T15 cells transfected with either control vector or CD38 expression vector was also assayed by ELISA.

Results: GIS from IH-treated HIT-T15 beta cells was significantly attenuated ($P < 0.05$), whereas that from the cells treated with normoxia was increased by high glucose. GIS from rat islets was also abolished by the treatment of IH ($P < 0.05$). The levels of insulin mRNAs in HIT-T15 cells and rat islets were unchanged. The mRNA levels of Glut2, GK, SUR1, and Cav1.2 in IH-treated islets were unchanged between IH-treated and normoxia-treated islets. We

then analyzed genes involved in Ca^{2+} mobilization from intracellular pools such as CD38, and found that the mRNA level of CD38 was significantly lower in IH-treated islets than in normoxia-treated islets (39% of the control). The reporter gene assay revealed that the transcription of CD38 was significantly attenuated by IH ($P < 0.01$), and the transfection of CD38 expression vector recovered the attenuation of GIS by IH ($P < 0.05$).

Conclusion: It is quite possible that the cyclic change of hypoxia-reoxygenation, which occurs in SAS patients, attenuates glucose-induced insulin secretion from pancreatic beta cells via CD38 down-regulation, resulting in glucose intolerance and type 2 diabetes.

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Involvement of the interaction between neuronal NO synthase and its protein inhibitor PIN in the control of insulin secretion

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Background and aims: We have previously shown that rat pancreatic β -cells express an isoform of neuronal NO synthase (nNOS) that controls insulin secretion through metabolic and non-metabolic activities. This latter activity is related to the interaction of nNOS with its protein inhibitor PIN at the level of insulin secretory granules. PIN is also the light chain of cytoskeletal motors like myosin V. Our aim is therefore to investigate the possible involvement of nNOS and PIN interaction in the control of glucose-induced insulin secretion.

Materials and methods: nNOS, PIN, and myosin V interaction was evidenced by immunoprecipitation and Western blotting in INS-1 cell extracts. nNOS-PIN interaction was analyzed using the Spot method consisting in the multiple synthesis of overlapping peptides on cellulose membranes. Insulin secretion was studied on INS-1 cells at low and stimulating glucose concentrations.

Results: In the INS-1 β -cell line, we could bring evidence for a direct interaction between nNOS, PIN, and myosin V by immunoprecipitation, confirming our previous immunofluorescence study. Moreover, PIN appeared to recruit myosin V as overexpression of PIN led to higher levels of immunoprecipitated myosin V. In order to set up tools to dissociate nNOS-PIN interaction, we analyzed the nNOS regions involved in this interaction, by performing the synthesis of peptides covering the nNOS sequence. We were able to identify two interacting zones: a strong affinity zone from amino acid 225 to 241 of nNOS and a zone of low affinity from residue 195 to 211. Mutational analysis performed on the high affinity zone allowed us to make out a peptide with three punctual mutations according to the native sequence, IDVGIQVDWD, that inhibits nNOS-PIN interaction with a K_i of 0.4 μM . This peptide, conjugated to the HIV sequence TAT to render it cell-permeant, was, at a 20 μM concentration, able to dissociate nNOS-PIN interaction by 47% as compared to a control peptide after a 7-hour pre-incubation period with INS-1 cells. At a more functional level, the peptide decreased insulin secretion induced by 5.6 mM glucose by 20% ($p < 0.05$), but did not modify insulin response in the presence of 2.8 mM glucose.

Conclusion: Our data strongly suggest that the nNOS-PIN interaction is involved in the modulation of insulin response to glucose, possibly at the level of the exocytotic machinery.

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The transcription factor ZBED6 is expressed in βTC6 and MIN6 cells and affects insulin production

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Background and aims: ZBED6 is a novel transcription factor derived from a domesticated DNA transposon. ZBED6 is exclusive to placental mammals and highly conserved among species. Silencing of ZBED6 in mouse C2C12 myoblasts affected IGF2 expression, cell proliferation, wound healing and myotube formation. Chromatin immunoprecipitation (ChIP) sequencing using C2C12 cells identified about 2,500 ZBED6 binding sites in the genome. Among these putative ZBED6 regulated genes, we found several genes that control pancreatic beta cell differentiation and function. The aim of the present study is to investigate the role of ZBED6 in beta cell function using the mouse βTC6 and MIN6 cell lines.

Materials and methods: Two stable ZBED6-shRNA βTC6 cell lines with different shRNA sequences (sh1 and sh2) were generated using lentivirus. The expression of ZBED6, Hif1- α , Pdx-1, Glucokinase, MafA and Insulin was examined by real-time PCR and/or immunoblotting. The subcellular localization of Pdx-1 was studied by immunofluorescence and confocal microscopy. For insulin content analysis, stable ZBED6-shRNA MIN6 cell lines were generated. Insulin release and content from MIN6 cells were measured by Meso Scale Discovery Insulin Assay Kit.

Results: ZBED6 expression was significantly knocked down at the protein level in both sh1 and sh2 βTC6 cell lines (sh1, 40 \pm 5%; sh2, 56 \pm 8%). As control an shMock lentiviral vector lacking ZBED6 shRNA sequencing was used. Morphological changes were observed in both sh1 and sh2 cell lines and it could be observed that the ZBED6 deficient cells formed islets-like clusters to a higher degree than control cells. Hif1- α expression was significantly down-regulated in sh1/2-cells while Pdx-1 was significantly up-regulated. Up-regulation of Pdx-1 was also confirmed by immunoblotting, and immunofluorescence staining showed nuclear localization of Pdx-1 in all βTC6 cell lines. Effects of ZBED6 knock down on Glucokinase, MafA and Insulin expression are listed in Table 1. Due to low insulin content of βTC6 cells, a stable ZBED6-sh1 MIN6 cell line was generated (49 \pm 7% ZBED6 mRNA vs shMock, $p < 0.05$). Similar morphological changes were observed in sh1 MIN6 cells as those observed in βTC6 cells. In addition, the insulin content of sh1 MIN6 cells was increased with 55% ($p < 0.05$) as compared to shMock MIN6 cells.

Conclusion: The transcription factor ZBED6 is expressed in beta cell lines and lowering of ZBED6 levels results in increased expression of beta-cell specific genes and augmented insulin contents.

	shMock βTC6	ZBED6-sh1 βTC6	ZBED6-sh2 βTC6
Zbed6	100%	25 \pm 3% *	41 \pm 5% *
Hif1- α	100%	56 \pm 6% *	68 \pm 3% *
Pdx-1	100%	265 \pm 43% *	186 \pm 22% *
Glucokinase	100%	263 \pm 69% *	121 \pm 28%
MafA	100%	391 \pm 128%	179 \pm 43%
Insulin	100%	2235 \pm 675% *	180 \pm 20% *

Table 1. Real-time PCR results from βTC6 cell lines. Results were normalized to GAPDH levels and expressed in percent of shMock. Mean \pm SEM, Student T-test, * $P < 0.05$ vs shMock, n=3-5

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Expression and function of CCL5 and GPR75 in islets of Langerhans

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Background and aims: G protein-coupled receptor 75 (GPR75) is a novel human GPCR that encodes a 540 amino-acid protein. Kidney cells transfected with a GPR75 plasmid show Gq-dependent elevations in intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) in response to chemokine (C-C motif) ligand 5 (CCL5), a 68 amino-acid chemokine. GPR75 does not share sequence homology nor ligand homogeneity with the chemokine receptor family, thus distinguishing it as an atypical chemokine receptor. We have recently amplified GPR75 mRNA in human and mouse islets, and found that GPR75 mRNA expression is significantly higher than that of other CCL5 receptors. This study was therefore aimed at investigating the expression and function of CCL5 and GPR75 in islets of Langerhans.

Materials and methods: CCL5 and GPR75 expression and cellular localisation were examined by Western blotting and immunohistochemistry. Changes in $[\text{Ca}^{2+}]_i$ were measured in mouse MIN6 β -cells by single cell Ca^{2+} microfluorimetry. Dynamic insulin secretion was quantified by radioimmunoassay after perfusion of isolated mouse and human islets. In *in vivo* experiments, mice were subjected to intraperitoneal glucose tolerance tests (IPGTTs) following a single administration of glucose (2g/kg body weight) in the absence or presence of 65pmol recombinant mouse CCL5.

Results: Immunohistochemistry demonstrated extensive GPR75 immunostaining of islet β -cells in mouse and human pancreas sections and this was confirmed by detection of a 59 kDa immunoreactive protein by Western blotting of islet proteins. CCL5 was also expressed by both mouse and human islets, although it was localised to insulin-expressing β -cells in human pancreas and to non- β -cells in mouse pancreas. Administration of recombinant mouse CCL5 induced rapid, reversible increases in $[\text{Ca}^{2+}]_i$ in Fura 2-loaded MIN6 cells (87 \pm 2%, 88 \pm 2% and 92 \pm 1% of the peak tolbutamide response at 0.25, 2.5 and 25nM CCL5 respectively, n=37). In addition, CCL5 significantly potentiated glucose-induced insulin secretion from mouse islets (20mM glu-

cose: $619 \pm 153\%$ basal; 20mM glucose+20nM mouse CCL5: $1559 \pm 456\%$ basal; $n=4$, $P<0.01$) and human CCL5 had similar stimulatory effects on insulin secretion from human islets. IPGTTs in lean mice showed that CCL5 reduced average blood glucose concentrations (CCL5 at 0, 15', 30', 60', 90' and 120 minutes after i.p. injection: 4.3 ± 0.2 , 16.5 ± 1.7 , 20.0 ± 2.4 , 14.5 ± 1.7 , 9.8 ± 0.9 and 7.4 ± 0.2 mM; $n=6$; control: 3.7 ± 0.2 , 21.6 ± 1.8 , 24.2 ± 1.4 , 18.3 ± 1.7 , 11.9 ± 0.7 and 7.6 ± 0.4 mM; $n=4$, $P<0.05$). Improvements in glucose tolerance in response to exogenous CCL5 were also observed in insulin-resistant ob/ob mice ($P<0.05$ vs controls at $t=15$ and 30min).

Conclusion: Both CCL5 and GPR75 are expressed by pancreatic islet cells with GPR75 predominantly expressed by insulin-secreting β -cells. Our results also indicate that exogenous CCL5 increases β -cell $[Ca^{2+}]_i$, stimulates insulin secretion from isolated mouse and human islets and improves glucose tolerance in vivo. These data suggest that CCL5 and/or GPR75 may be novel targets for the treatment of type 2 diabetes.

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The complement inhibitor protectin influences insulin secretion and is correlated to glucose intolerance in humans

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Background and aims: Complement activation is known to be involved in the pathology of type 1 diabetes but little is known about the role of complement in type 2 diabetes even though complement genes are expressed in the pancreatic islets. Gene expression data showed that of all complement-related genes, the complement inhibitor protectin (CD59) was most highly expressed in human and rodent islets. Protectin is a cell surface GPI linked protein, which main function is to prevent complement-mediated cell lysis. In this study we aimed to investigate the role of protectin in terms of insulin secretion and expression in healthy and diabetic human islets, as well as in rat islets and clonal beta-cells.

Materials and methods: Gene expression in Wistar and Goto-Kakizaki (GK) rat islets and INS1 832/13 cells: cDNA from isolated rat islets and INS1-832/13 cells was analyzed for gene expression using Q-PCR. Gene expression in human islets: Total RNA was isolated from pancreatic islets from 80 donors using the AllPrep DNA/RNA Mini Kit (Qiagen) and analyzed using Gene 1.0 ST whole transcript based assays (Affymetrix). Knockdown of protectin: INS-1 832/13 cells were transfected with 30 nM siRNA directed against rat protectin (Ambion) using Dharmafect (Dharmacon). Knockdown was verified with Q-PCR after 48h. Reduction in surface protectin after siRNA treatment was confirmed by flow cytometry. Insulin secretion assay in clonal beta-cells: 72h after siRNA transfection, INS1-832/13 cells were preincubated for 2 hours in 2.8 mM glucose and then stimulated with 2.8 or 16.7 mM glucose for one hour. Insulin secretion in human islets: Islets were preincubated for 30 min in KRB with 1 mM glucose in presence or absence of protectin antibody and then stimulated with 1 or 16.7 mM glucose for one hour.

Results: Gene expression of protectin in the diabetic GK rat was significantly lower than in the healthy Wistar rat, 0.52 relative HPRT compared to 1.4 ($p<0.05$). To mimic this effect, we used siRNA to silence protectin in clonal beta-cells. Treatment with siRNA reduced mRNA expression with 85% ($p=0.001$). Protein levels were decreased with approximately 50% as verified by flow cytometry. Silencing of protectin caused a 50% reduction in glucose-stimulated insulin secretion ($p<0.05$). In human islets gene expression of protectin was significantly increased in individuals with high HbA1c ≥ 6.0 ($n=23$) compared to healthy individuals with HbA1c ≤ 5.5 ($n=27$) ($p=0.01$). Blocking the complement-inhibitory site on protectin with a monoclonal antibody led to a tendency of an increase in insulin secretion in healthy human islets, whereas it caused a tendency to a decrease of insulin secretion in diabetic islets.

Conclusion: We have found that the complement inhibitor protectin is important for insulin secretion, and is regulated in response to high HbA1c. We believe that the decreased expression in GK rat is caused by genetic factors, whereas the increased expression in diabetic humans is a compensatory response to maintain functional insulin secretion. The stimulatory effect on insulin secretion seen in human islets when the known functional site is blocked indicates that protectin has other yet unknown signalling properties. *Supported by: NN foundation, Swedish Research Council, NNCIT*

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Serotonin (5-HT) receptor expression in human islets of Langerhans

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Background and aims: Regulation of insulin secretion is pivotal to maintain overall glucose homeostasis. When insulin secretion fails due to a complex interaction between genes and environment, type 2 diabetes prevails. Serotonin (5-HT) and its receptors have been suggested to play an active role in β -cell expansion during pregnancy in rodents. 5-HT is produced in β -cells, stored in insulin granules and thus co-released with insulin upon stimulation. 5-HT receptors belong to a diverse group of G-protein coupled receptors and ligand gated ion channels causing transient or persistent intracellular changes depending on the type of receptor activated. The presence of 5-HT receptors in the human endocrine pancreas remains a largely unexplored area. Therefore, in this study, this issue is further investigated.

Materials and methods: Human islets from 64 islet donors were provided in collaboration with the Nordic Network for Clinical Transplantation, Sweden. mRNA was isolated from untreated islets and microarray with the Affymetrix TM Gene 1.0 ST Whole Transcript Assay was performed and normalized by RMA normalization. Logistic regression was used to correlate between gene expression, metabolic parameters, gender and purity.

Results: Human islets of Langerhans express low levels of mRNA for 17 different serotonin receptors. Twelve of these receptors belong to the G-protein coupled receptor family, but expression for all members of the voltage-gated ion channel 5-HTR3 receptor family was also observed. 5-HTR2A mRNA levels were significantly correlated to HbA1c ($\beta=0.2888$, $P=0.038$). Donor body mass index (BMI) was significantly negatively correlated to 5-HTR3E ($\beta=-0.252$, $P=0.044$) and 5-HTR4 ($\beta=-0.314$, $P=0.011$). Furthermore, male donors had significantly higher expression levels of 5-HTR3C ($P=0.01$) and 6 (78.8 ± 10.7 vs 69.4 ± 9.4 , $P<0.000$) receptors than female donors. Islet purity was positively correlated to expression of 5-HTR1F ($\beta=0.373$, $P=0.009$) and negatively to 5-HTR5A ($\beta=-0.250$, $P=0.046$).

Conclusion: Seventeen different 5-HT receptors are expressed in human islets of Langerhans. As serotonin is expressed and co-released with insulin, serotonin and its receptors could modulate hormone secretion from various cell types within the human islet of Langerhans.

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Knockdown of 5-HT2b receptor decreases glucose stimulated insulin secretion in a clonal (INS (832/13) beta cell line

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Background and aims: 5-Hydroxytryptamine (5-HT), also known as serotonin activates a large family of receptors. 5-HT1a, 5-HT2b and 5-HT1d have all previously been shown to be expressed in islets of Langerhans from rodents. All three receptors are G-protein coupled receptors but activation result in different cellular events due to activation of different G-proteins. The amine 5-HT has been shown to be present and co-secreted with insulin from pancreatic beta-cells. As both receptors and amines are present within the islets, serotonin may potentially regulate hormone secretion from islets of Langerhans. We have previously investigated activation of 5-HT2b using a selective 5-HT2b agonist (alpha-Methyl serotonin) which potentiates glucose stimulated insulin secretion in the clonal beta-cell line, INS-1 (832/13). These experiments have also been performed in primary cells; mouse and human islets of Langerhans, with the same result. In this study we investigate the effect on insulin secretion in INS-1 (832/13) cells by performing specific knock down of the receptor.

Materials and methods: We used RT PCR and sequence specific primers to detected expression of the receptors in the beta-cell line (INS 832/13) and in islets of Langerhans. Immuno-histochemical analysis was used to detect the receptors at the protein level in rodent islet and human islets. We used Q (Quantitative) PCR and sequence specific primers to detect expression of the receptor in the INS-1 (832/13) cell line. Transfection was performed with RNA interference and Lipofectamine 2000. The knock down was verified by QPCR using specific primers and western blot.

Results: 5-HT2b mRNA was found in the 832/13 cells. We also found both 5-HT1a and 5-HT2b to be expressed in rodent and human islets, at both mRNA and protein level. Interestingly, 5-HT receptors in islets were localized in two different cell types in the islets. In rodent islets, 5-HT2b was predominantly expressed in beta-cells while 5-HT1a was more abundant in the alpha-cells. In human, islets the situation was reversed. The expression of the receptor was determined by QPCR and showed a robust expression in the INS-1 (832/13) cells. Specific knock down of the 5-HT receptor was achieved with -50% and verified by q-PCR and western blot. When cells treated cells were stimulated with high glucose, a significant decrease in insulin secretion at stimulatory concentrations of glucose (16.7 mM) was observed as compared to controls.

Conclusion: Our previous experiments show that an activation of 5-HT2b receptor using alpha-methyl serotonin potentiates glucose stimulated insulin secretion. Conversely, in knock down experiments, cells with a decreased expression of the 5-HT2b receptors display a perturbation of glucose stimulated insulin secretion. Therefore, our results strongly suggest that the 5-HT2b receptor plays a pivotal role in regulation of glucose stimulated insulin secretion.

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High GADA titre: a marker of insulin dependence in adult onset autoimmune diabetes

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Background and aims: In latent autoimmune diabetes of adults (LADA), the progression to insulin-dependent diabetes is usually faster than in type 2 diabetes but the factors influencing this progression are not completely understood. The aim of the present study was to determine whether GADA titre could define the risk of progression to insulin therapy in LADA patients. We also evaluated other parameters (gender, BMI, TPO and IA-2 antibodies) associated with early development of insulin dependence. Adult onset GADA positive autoimmune diabetes subjects (n=191) were selected from the Non Insulin Requiring Autoimmune Diabetes (NIRAD) study cohort of 4,250 type 2 diabetes subjects. The analysis of GADA titre showed a bimodal distribution which identified two subgroups of patients with high (>32 GADA U) and low GADA titre (≤32 GADA U).

Materials and methods: One hundred ninety GADA positive patients were followed for 6 years from diagnosis to evaluate the progression of patients started insulin therapy. Kaplan-Meier curves were plotted and log-rank test was performed to identify possible markers capable to influence the progression into insulin dependence. During the follow up, 6 patients out of 191 GADA positive dropped out from the study.

Results: The number of GADA positive autoimmune diabetes patients who required insulin therapy was 93/191 (48.7%). We observed that a significant higher number of high GADA titre patients 61/93 (65.6%) progressed to insulin dependence within 6 years of diagnosis compared to low GADA titre patients 32/93 (34.4%) (p=0.003). Furthermore, GADA positive autoimmune diabetes patients with BMI<25 kg/m² had a faster progression, although not statistically significant, towards insulin therapy compared to patients with BMI ≥ 25 kg/m² (p = 0.07). Finally, we observed that gender, TPO and IA-2 Ab titre were not predictive markers of progression into insulin dependence.

Conclusion: High GADA titre is a marker of progression towards insulin dependence in LADA patients.

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Influence of HLA genes on antibody reactivity to the juxtamembrane and central region epitopes of the autoantigen IA-2

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Background and aims: The HLA locus is the major genetic determinant of susceptibility to Type 1 diabetes, with 95% of Caucasian Type 1 diabetic patients expressing HLA-DR3, HLA-DR4 or linked HLA-DQ alleles. Knowledge of the relationships between HLA gene expression and immune responses to islet antigens in Type 1 diabetes is important for the development of individualised, targeted immune intervention. IA-2 is a major autoantigen in Type 1 diabetes, and antibody responses to the molecule have been shown to be weakly associated with HLA-DR4. Several specific regions of antibody reactivity to the IA-2 protein have been identified, including two linear epitopes within the juxtamembrane domain (JM1 and JM2) and a major conformational epitope within the 831-860 region that is represented by a central region IA-2 construct (643-937). The aim of this study was to further define the relationship of HLA gene expression to the presence of antibodies to these specific epitopes on the IA-2 autoantigen.

Materials and methods: Serum samples were obtained from 110 Type 1 diabetic patients within 6 months of diagnosis and studied for the presence of autoantibodies to IA-2 constructs representing the cytoplasmic region of IA-2 (IA2ic; 604 - 979), and to the central region (643 - 937) and juxtamembrane (605 - 693) domains of IA-2 by radioligand binding assays. Antibodies to specific JM1 (601 - 620) and JM2 (621 - 640) epitopes within the juxtamembrane domain were detected by determining blocking effects of peptides

corresponding to the amino acid sequences of these linear epitopes. HLA genotyping for DR and DQ alleles was performed by PCR with sequence-specific primers.

Results: Antibodies to the cytoplasmic domain of IA-2 were detected in 75% of HLA-DR3/4 heterozygous patients, in 67% of HLA-DR4/non-DR3 patients and in 63% of those negative for HLA-DR4. Antibodies to JM domain epitopes were detected in 37% of HLA-DR4-positive patients, but in only 7% of those lacking HLA-DR4 ($p < 0.01$). There was a similar association of HLA-DR4 expression with antibodies to central region epitopes (HLA-DR4-positive 44%; non-DR4 20%; $p < 0.05$). In HLA-DR4 subjects, juxtamembrane domain antibody reactivity was predominantly to the JM2 epitope. The frequencies of antibodies to JM and central region epitopes were similar in HLA-DR4-DQ8 and HLA-DR4-DQ7 patients, indicating that the HLA association is with primarily with the DR rather than DQ locus.

Conclusion: Analysis of antibodies to specific epitopes on the IA-2 molecule identifies immune reactivity more strongly associated with HLA-DR4 expression than antibody reactivity to the whole molecule. Furthermore, the low frequency of antibodies to JM and central region epitopes in HLA-DR4-negative subjects (27%), despite a high prevalence of antibodies to IA-2ic (63%), is indicative of an unidentified epitope linked to a non-HLA-DR4 allele. Hence, studies of immune reactivity to specific epitopes on autoantigens will help determine the role of HLA genes in regulating autoimmune responses in type 1 diabetes.

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Investigation of the HLA-B39 epitopes for preproinsulin, GAD65/67, IA-2 and IA-2B (phogrin)

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Background and aims: It has been shown that the highest-risk allelic combination of type 1 diabetes, (HLA-DR3/4), in the presence of HLA-B39 exhibits marked acceleration of disease onset, from the point of detection of the first biochemically-defined diabetes-related autoantibody. We wished to investigate the bases for this property, by scanning the major type 1 diabetes autoantigens for possible peptides that could be recognized by cytotoxic T cells restricted to HLA-B39.

Materials and methods: The aminoacid sequences of four diabetes-related autoantigens (preproinsulin, GAD65, IA-2 and IA-2b (phogrin), the last two in their antigenic regions) were scanned for the presence of motifs for HLA-B39. We limited ourselves to alleles B3901 and 3906, already known to be present within the studied population in proportion of 83.3 % to 16.7 % respectively. The motif for HLA-B3901 was obtained from the data base www.syfpeithi.de while that for -B3906 was deduced by molecular simulation based on the crystal structure of HLA-B3501 to have identical B, F anchors. We recorded both strong epitopes, and weak ones (strong anchors at pocket B, weak ones at pocket F). We considered lysine at pocket B as rendering a strong motif, in contrast to the prediction of the data base. Upon scanning we performed molecular simulation studies of several epitopes from the above autoantigens bound to B3901. We included GAD67 in the scan in order to determine if there were specificities of the one isoform versus the other regarding the HLA-B3901 allele.

Results: The four autoantigens are replete with epitopes to HLA-B3901/3906, both strong as well as weak ones, from octamers to endecamers. The epitopes, both strong and weak, cover the entire autoantigen sequences indicating that there seems to be no selectivity. The following summary of results indicates the extent of reactivities:

autoantigen	Weak epitopes	strong epitopes	total aminoacid sequence scanned
Preproinsulin	5	9	110
GAD65	15	23	585
GAD67	9	19	594
IA-2	31	39	379
IA-2b	18	33	376

There are a number of cases where a given aminoacid stretch contains overlapping octamers to endecamers, with identical pocket B anchor. Of special interest are the cases where strong and weak epitopes are present in regions reactive with specific autoantibodies, as in the regions 250-295 and 521-575 of GAD65, and the region of B4-C10 of proinsulin. The molecular simulation results of a number of these interesting epitopes in complex with HLA-B3901 demonstrate very good fit into the groove of B3901.

Conclusion: One intriguing hypothesis, which must be tested experimentally, for the HLA-B39 acceleration, concerns the possibility of autoantibody-mediated antigen presentation via the class I MHC pathway, to explain the several epitopes in regions recognised by the autoantibodies.

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Decreased levels of memory T cells and increased levels of plasmacytoid dendritic cells in type 1 diabetes patients with short disease duration

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Background and aims: In type 1 diabetes (T1D), a T cell mediated autoimmune disease, both, the adaptive as well as the innate immune system are involved in destruction of β -cells. The aim of this study was to elucidate the frequency of peripheral innate and adaptive immune cells as well as regulatory cells in the blood of T1D patients with short disease duration in comparison to healthy individuals.

Materials and methods: Peripheral blood from 38 T1D patients with disease duration < 5 years with a median age 21 years (interquartile range: 15-39) and 38 healthy individuals with a median age of 26 years (23-29) was stained with fluorochrome conjugated antibodies and a multi-parameter FACS analysis was performed to quantify the following immune cells: CD4⁺CD25^{high}FoxP3⁺CD127^{dim} Tregs, memory and naïve subpopulations of CD4⁺ and CD8⁺ T cells, NK, NKT, myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC). The total number of neutrophil, basophil and eosinophil granulocytes as well as monocytes was measured by the use of a haematological cell counter.

Results: The percentage of CD4⁺ memory T cells within leukocytes was significantly decreased in patients compared to controls [median+interquartile range: 6.5 (5.3-7.3)% vs. 7.5 (6.8-8.8)%, $p < 0.001$]. CD8⁺ memory T cells were also decreased in patients [1.9 (1.4-2.7)%] compared to controls [2.6 (2.0-3.4)%, $p = 0.003$]. Furthermore pDC were increased in the Lin CD45⁺DR⁺ population in the T1D group compared to healthy subjects [36.4 (32.5-43.7)% vs. 31.1 (25.7-36.3)%] whereas mDC were equally distributed in both groups. The frequency of peripheral Tregs, whole population of CD4⁺ and CD8⁺ T cells, NK, NKT, mDC and all subtypes of granulocytes and monocytes were similar between T1D patients and healthy controls. Levels of C-reactive protein were within the normal range in both investigated groups.

Conclusion: The results of this study show clear differences in important subtypes of peripheral T cells and pDC but no alteration in the frequency of regulatory T cells in T1D patients with short disease duration. Further studies are needed to elucidate the impact of the observed changes in the frequency of memory T cells and pDC in the pathogenesis of T1D.

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High risk vs low risk nondiabetic first degree relatives of type 1 diabetics: differences in CXCR3+, CCR4+ and CD25hi T memory cells

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Background and aims: It has been previously suggested that changes in T memory lymphocyte subsets might be associated with the onset of Type 1 diabetes (T1D). Simultaneously, the expression of Th1 and Th2 associated chemokine receptors determine the recruitment of CD4⁺T cells into sites of inflammation, and the disturbance in T regulatory (T reg) subsets, contribute together to the onset of T1D. However, the relevance of the changes in expression of chemokine receptors CXCR3, CCR4, and CD25hi on T memory cells, for the development of T1D has not yet been elucidated. Therefore, the aim of this study was to compare the changes in percentage of (a) CXCR3+

(Th1-associated) (b) CCR4+ (Th2 associated) and (c) CD25hi (T reg associated) subsets of T memory cells, between two groups, the high-risk and the low-risk group, of nondiabetic first-degree relatives (FDRs) of patients with T1D as well as in the group of healthy controls. The difference between the two groups of FDRs was based on presence or absence of glutamic acid decarboxylase (GADA) and tyrosine phosphatase insulinoma antigen-2 (IA-2) antibodies. Thus, in the study we included 12 high-risk nondiabetic FDRs (GADA+, IA-2+) (group A) and 37 low-risk nondiabetic FDRs (GADA-, IA-2-) (group B) and 20 healthy unrelated control subjects.

Materials and methods: T1D and glucose intolerance were excluded in the study by using WHO criteria. GADA and IA-2 levels were determined by ELISA. The percentages of CXCR3+, CCR4+ and CD25hi T memory cell subsets were analyzed in peripheral blood by using four-color immunofluorescence staining and flowcytometry.

Results: When the percentage of CXCR3+ T memory cells was analyzed, it was found to be higher in group A vs groups B and C (A: 63.25 ± 6.52 vs B: 51.83 ± 6.77 ; C: 52.89 ± 6.25 %, $p < 0.05$). In contrast, the percentage of CCR4+ T memory cells was significantly lower in group A vs groups B and C (A: 30.02 ± 3.20 vs B: 41.48 ± 8.54 ; C: 40.65 ± 7.20 %, $p < 0.05$). Simultaneously, the percentage of CD25hi T memory cells was significantly lower in group A vs groups B and C (A: 0.16 ± 0.03 vs B: 0.26 ± 0.01 ; C: 0.26 ± 0.06 %, $p < 0.05$).

Conclusion: Our results have demonstrated that high risk FDRs showed higher levels of CXCR3+ T memory cells associated with increased Th1 response, together with lower levels of CCR4+ Th2 and CD25hi T reg cells subsets. The results imply that in FDRs the risk for developing T1D might be associated with enhanced activity of Th1 and diminished activity of Th2 and T regulatory immune response.

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HLA- and autoantibody-associated T-cell responses to IA-2 determinants in type 1 diabetes

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Background and aims: Type 1 diabetes is associated with expression of HLA-DR3, DR4, DQ2 and DQ8, but the manner by which these gene products influence autoimmune responses to pancreatic islet antigens is poorly understood. HLA-DR4 associations have been observed for antibodies to IA-2, but this is likely to reflect a primary association with T-cell responses. The aim of this study is to identify T-cell responses to specific IA-2 peptides that are associated with HLA-DR4 and linked to antibody responses to specific regions of IA-2.

Materials and methods: Peripheral blood lymphocytes were obtained from 26 Type 1 diabetic patients aged 12–29 years within 6 months of disease onset. Frequencies of T-cells secreting the cytokines IFN- γ , IL-10 and IL-17 in response to 7 peptides previously shown to represent major T-cell determinants in diabetes were determined by ELISPOT and expressed as a stimulation index (SI) relative to numbers of cytokine-secreting cells in medium alone. Responses with SI > 3 were considered positive. Patients were typed for HLA-DR and DQ alleles by PCR using sequence-specific primers. Sera from the same patients were analysed for autoantibodies to constructs representing the cytoplasmic (605–979), juxtamembrane (605–693), tyrosine phosphatase (643–979) and central region (643–937) domains of IA-2 by radioligand binding assay.

Results: Positive IL-10 responses were detected to all 7 peptides tested, but strongest SIs (4–19) were observed to peptides representing 831–850, 841–860, 853–872 and 955–976 regions of IA-2. Positive responses to at least one of these peptides were observed in 11/17 (65%) patients expressing HLA-DR4, but were absent in non-DR4 patients ($p < 0.05$). IL-17 responses to the peptides tested were rare, but were also detected exclusively in DR4-positive patients, and 5/9 of responses detected were accompanied by an IL-10 response to the same peptide. Co-incubation of cells with IL-1 β , that is known to promote differentiation of Th17 cells, specifically increased peptide-stimulated IL-17 responses. The proportion of patients showing IFN- γ responses to the selected peptides were low (23%), and these were detected at similar frequencies in HLA-DR4-positive and negative individuals. Significantly higher IL-10 responses to peptides 841–860 and 853–872 were observed in patients positive for antibodies to the central region construct ($p < 0.05$) that harbours an epitope overlapping the 841–872 region. IFN- γ responses were not associated with autoantibodies.

Conclusion: T-cells responding to IA-2 determinants in HLA-DR4-positive diabetic patients are characterised by secretion of the regulatory cytokine IL-

10, but also include cells secreting IL-17, supporting a role for Th17 cells in disease pathogenesis. The balance may be shifted towards secretion of the pro-inflammatory cytokine by the presence of IL-1 β , as may occur in an insulinitis. The association of T-cell responses to specific determinants with presence of antibodies to an overlapping epitope supports a role for T-B collaboration in autoimmunity in Type 1 diabetes.

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Membrane and intracellular expression of CTLA-4 in T regulatory lymphocytes (Tregs) and monocytes of healthy subjects and patients with type 1 diabetes: similarities, differences and correlations

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Background and aims: CTLA-4 (Cytotoxic T Lymphocyte Antigen-4, ITIM12) is genetically linked to type 1 diabetes and is thought to be one of the major suppressive molecules of Tregs, acting as a negative regulator at the immune synapse between antigen-presenting cells and T effector cells. The aim of our study was to investigate the membrane and intracellular expression of CTLA-4 in Tregs and monocytes of type 1 diabetes patients and healthy controls.

Materials and methods: Peripheral blood from 13 newly-diagnosed (nd) type 1 diabetics (9M/4F, ages 12.5 ± 9.4 years), 26 long standing (ls) patients (12 M/14F, ages 26.7 ± 9.2 years) with mean disease duration of 11.6 ± 7.4 years and 32 healthy controls (c) with no first or second degree relatives suffering from any autoimmune disease (13M/19F, ages 25.3 ± 11 years) was analysed by triple colour flow cytometry for the mCTLA-4 (membrane) and icCTLA-4 (intracellular) expression in Tregs and monocytes.

Results: There is a very significant deficiency in the amount of Tregs at the disease onset, which is only partly corrected in long standing patients. CTLA-4 is clearly expressed both in the membrane and the cytoplasm of Tregs without differences between type 1 diabetes patients and controls (nd: mCTLA-4 % 26.20 ± 28.33 MFI (Mean Fluorescence Intensity - number of molecules per cell) 129.18 ± 151.48 , icCTLA-4 % 44.86 ± 37.70 MFI 449.89 ± 282.60 , ls: mCTLA-4 % 14.97 ± 18.84 MFI 172.33 ± 158.68 , icCTLA-4 % 44.02 ± 44.51 MFI 350.81 ± 280.78 , c: mCTLA-4 % 19.23 ± 26.02 MFI 178.73 ± 140.94 , icCTLA-4 % 37.93 ± 46.79 MFI 450.18 ± 598.00) (in all comparisons $p > 0.05$). CTLA-4 is also clearly expressed both in the membrane and the cytoplasm of monocytes with significantly lower percent of membrane expression in long standing diabetics compared to controls ($p = 0.007$). (nd: mCTLA-4 % 25.31 ± 28.89 MFI 154.36 ± 110.20 , icCTLA-4 % 47.64 ± 44.30 MFI 848.75 ± 709.48 , ls: mCTLA-4 % 8.90 ± 6.49 MFI 274.36 ± 295.60 , icCTLA-4 % 56.18 ± 45.56 MFI 885.08 ± 707.15 , c: mCTLA-4 % 23.67 ± 27.17 MFI 238.21 ± 242.69 , icCTLA-4 % 40.27 ± 47.79 MFI 902.28 ± 660.02) (in all other comparisons $p > 0.05$). There are highly statistically significant positive correlations of the frequency and intensity of CTLA-4 expression between Tregs and monocytes both in newly-diagnosed type 1 diabetics (mCTLA-4 % $r = 0.553$, $p = 0.05$ MFI $r = 0.803$, $p = 0.001$, icCTLA-4 % $r = 0.935$, $p < 0.001$ MFI $r = 0.746$, $p = 0.005$) and controls (mCTLA-4 % $r = 0.396$, $p = 0.03$, icCTLA-4 % $r = 0.95$, $p < 0.001$ MFI $r = 0.558$, $p = 0.003$), which partially change in long standing patients (mCTLA-4 % $r = 0.215$, $p = 0.302$ MFI $r = 0.328$ $p = 0.110$, icCTLA-4 % $r = 0.732$, $p < 0.001$ MFI $r = -0.537$, $p = 0.012$).

Conclusion: The significant membrane and intracellular expression of CTLA-4 in Tregs and monocytes confirms the importance of this molecule in the mechanisms of peripheral tolerance. The highly correlated expression of CTLA-4 between Tregs and monocytes of healthy controls and newly-diagnosed type 1 diabetes patients, which partially change in long standing patients, should be further investigated.

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Low frequency of T regulatory lymphocytes in children under 5 years old with newly recognised type 1 diabetesA. Szypowska¹, A. Stelmaszyk-Emmel¹, U. Demkow¹, D. Witkowski², W. Feleszko¹, W. Łuczyński³;¹Medical University of Warsaw, ²The Children's Memorial Health Institute, Warsaw, ³Medical University of Białystok, Poland.

Background and aims: The incidence of type 1 diabetes (T1D) in children has been increasing worldwide, with the greatest increase in children <5 years old. Previous observations pointed to critical role for T regulatory cells (Tregs) in the maintenance of self tolerance and the pathogenesis of diabetes. The suppressive capacity of Tregs is mainly contact-dependent but also based on immunosuppressive cytokines production: interleukin-10 (IL-10) and transforming growth factor-beta (TGF-beta). There are no studies focused on Tregs in children <5 yrs with T1D. The aim of the study was to analyze different subpopulations of Tregs in children with newly recognized T1D.

Materials and methods: 80 children <18 yrs. old were enrolled to the study, 40 subjects with newly recognized T1D and 40 healthy controls. Children with T1D were divided into two groups: 20 children <5 yrs (mean age: 2.8±1.1 yrs) and 20 children >5 yrs (mean age: 12.9±3.4 yrs). The control group consisted of 20 healthy children <5 yrs (mean age: 2.6±1.0 yrs) and 20 children >5 yrs (mean age 10.7±3.7 yrs). Flow cytometric analysis of Tregs was performed in blood samples from all children. The intra- and extracellular proteins were evaluated using monoclonal antibodies to the following markers: CD4, CD25, CD127, Foxp3, IL-10, and TGF-β. The following data were collected: morphology, fasting C-peptide, ICA, GADA, IA-2A.

Results: There was significantly lower percentage of CD4+CD25highCD127lowFoxp3+ cells in children <5 yrs with T1D compared to children >5 yrs with T1D (median 0.865% vs. 1.555%, respectively, $p=0.017$). There was the lower frequency of CD4+CD25highCD127lowFoxp3+ cells in children >5 yrs with T1D compared to healthy controls (median 1.555% vs. 3.44%, respectively, $p=0.0009$). The percentages of the following subpopulation of lymphocytes was lower in children <5 yrs with T1D compared to age matched healthy controls: CD4+CD25highCD127lowFoxp3+ (median 0.865% vs. 4.53%, $p<0.0001$); CD4+CD25highTGF-β (median 0.93% vs. 1.74%, $p=0.049$); CD4+CD25highIL-10 (mean 1.15 ± 1.16% vs. 1.96 ± 1.38%, $p=0.036$). There was no significant difference in the following populations of Tregs between children >5 yrs with T1D and age matched healthy controls CD4+CD25highTGF-β (median 1.46% vs. 1.05%, $p=0.616$); CD4+CD25highIL-10 (median 1.39% vs. 0.96, $p=0.620$). There was no significant difference in the following populations of Tregs between children <5 yrs and >5 yrs with T1D: CD4+CD25highTGF-β (1.74% vs. 1.46%, $p=0.104$), CD4+CD25highIL-10 (median 0.87% vs. 1.29%, $p=0.328$). Children <5 yrs with T1D had the lower C-peptide level compared to children >5 yrs with T1D (median 0.32 ng/ml vs. 0.80 ng/ml, $p=0.0005$).

Conclusion: Children <5 yrs. with newly recognized T1D showed more significant changes in different subpopulations of Tregs compared to children >5 yrs with T1D that might reflect the influence of more aggressive beta cells destruction and T1D onset at a younger age. Further clinical studies investigating the regulatory T cells in the large cohort of children with type 1 diabetes are needed.

PS 017 Stem cell and islet cell proliferation: expanding knowledge and new developments

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Role of ductal cell dedifferentiation on new beta cell formation in the post-natal rat pancreas undergoing regeneration after partial pancreatectomyN. Tellez^{1,2}, M. Vilaseca^{1,2}, J. Escoriza^{1,2}, E. Montanya Mias^{1,2};¹Lab. Diabetes and Experimental Endocrinology, IDIBELL-University of Barcelona, L'Hospitalet de Llobregat, ²CIBERDEM, Barcelona, Spain.

Background and aim: Partial pancreatectomy (Px), a well defined model of pancreatic regeneration, induces profound changes in tissue composition and cell phenotype. Specifically, areas composed of metaplastic ducts that have been proposed to harbour progenitor cells appear immediately after surgery. In a recent study, we found that beta cell neogenesis was stimulated in Px rats treated with gastrin (Px+G). The aim of the study was to investigate whether key aspects of beta cell neogenesis could be identified by comparing the endocrine regeneration events that take place in Px and Px+G rat pancreases.

Material and methods: Sprague-Dawley rats underwent 90%-Px and were treated with [15leu] gastrin-17 (Px+G, n=19) or with vehicle (Px+V; n=20). Pancreatic remnants were harvested on days 1 and 3 after Px and processed for total RNA extraction or paraffin embedding. Gene expression was determined by quantitative real time PCR and protein identification by immunofluorescence.

Results: Blood glucose levels were similar between both Px groups (Px+V: 126±10 mg/dl; Px+G: 110±8 mg/dl). Beta cell mass was significantly increased in Px+G rats 72h following Px (Px+V: 0.28±0.076 mg, Px+G: 0.57±0.11 mg; $p<0.05$), but beta cell replication and apoptosis were similar in both Px groups (Px+V: 3.92±0.44%, Px+G: 3.20±0.31%; $p=ns$ for beta cell replication and Px+V: 0.59±0.23%, Px+G: 0.37±0.20%; $p=ns$ for beta cell apoptosis). The number of small beta cell clusters (< 5 insulin-positive cells), an indirect marker of beta cell neogenesis, was increased in Px+G remnants, suggesting that the higher beta cell mass in Px+G group was attributable to increased beta cell neogenesis. Gene expression of pro-endocrine transcription factors: neurog3, neuroD1, pdx-1 and nkx6.1 was upregulated, and ductal cell markers ck20, ca2, hnf1β, hnf6 and prominin1 downregulated in Px+G remnants compared with Px+V ($p<0.05$). Initially after pancreatectomy regions of metaplastic ducts surrounded by mesenchymal cells are formed. The relative volume of these areas of regeneration was similar in both groups (Px+V: 38±7.05% and Px+G: 35.1±5.68%). Metaplastic ducts had lower immunoreactivity for CK20 and 41.7±0.7% of these cells exhibited low nuclear expression of the key endocrine differentiation marker NKX6.1 which is beta cell specific in the post-natal pancreas. In Px+G remnants 57.6±3.9% of metaplastic ductal cells expressed low levels of NKX6.1 ($p<0.05$ vs Px+V).

Conclusion: This study supports the concept that after 90% Px, new beta cells are formed by neogenesis that contribute to the regeneration of the beta cell mass. The enhanced ductal cell plasticity in Px rats treated with gastrin, illustrated by downregulation of ductal cell markers and higher percentage of ductal cells expressing the endocrine differentiation marker NKX6.1, suggests that cell dedifferentiation / redifferentiation processes play a role on new beta cell formation in the post-natal rat pancreas after partial pancreatectomy.

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TCF7L2 plays a role in beta cell regenerationK. Maedler¹, J. Kerr-Conte², L. Shu¹;¹Centre for Biomolecular Interactions, University of Bremen, Germany,²Centre for Biomolecular Interactions, Thérapie Cellulaire du Diabète, INSERM / Université de Lille, France.

Background and aims: Both type 1 and type 2 diabetes are characterized by a loss and dysfunction of the β-cell. A major goal of diabetes therapy is to promote the formation of new β-cells. Polymorphisms of TCF7L2, a transcription factor of the Wnt signaling pathway, have been reported to be associated with T2DM, regulating β-cell survival and function. Pancreatic duct cells are considered a potential source of β-cell regeneration. Here we provide a possible role for TCF7L2 in β-cell regeneration from pancreatic ductal epithelial cells.

Materials and methods: Pancreatic sections from three mouse models (high fat/high sucrose fed mice: HFD, Exendin-4: Ex-4 10nmol/kg and streptozotocin: STZ, 90 mg/kg -treated mice) and from healthy individuals and patients with T2DM were used to investigate the association of β -cell regeneration and TCF7L2 expression by immunostaining of insulin, CK19, TCF7L2, Ki67 and Pdx-1. TCF7L2 was overexpressed in isolated human pancreatic exocrine cells and immunostaining and RT-PCR analysis for insulin, CK19, Ki67, Pdx-1 and ngn3 were performed to investigate the effect of TCF7L2 on duct cell to β -cell conversion.

Results: We found increased intra-islet TCF7L2 expression in STZ- and Ex-4 treated mice, compared to controls (49.65% in STZ- and 40.9% in Ex-4-treated compared to 24.7% TCF7L2 positive β -cells in control mice, $p < 0.05$). Increased TCF7L2 expression was also observed in HFD treated mice, but only after 4 and 8 weeks of feeding (26.3% TCF7L2 positive β -cells in ND control mice, 42.8% positive β -cells after 4 weeks and 36.25% positive β -cells after 8 weeks HFD feeding, $p < 0.05$), in contrast, intra-islet TCF7L2 expression decreased after 12 weeks of HFD feeding (18.8% TCF7L2 positive β -cells after 12 weeks and 9.8% after 16 weeks HFD, $p < 0.05$), which occurred in parallel with the development of β -cell failure, suggesting that expression levels of TCF7L2 correlate with β -cell de-compensation and the progression of diabetes. Increased TCF7L2 expression in ductal epithelial cells was observed in STZ- and Ex-4 treated mice (68.3% TCF7L2 positive ductal cells in STZ- and 56% in Ex-4-treated mice, compared to 32.21% in control mice, $p < 0.005$). An increased number in Pdx-1 positive ductal cells were found in these mice; 5.3% Pdx-1 positive CK19 positive cells were found in STZ-treated mice and 3.3% in Ex-4 treated mice, compared to 0.7% in the control mice ($p < 0.005$). Together with the appearance Pdx-1 positive ductal epithelial cells in STZ- and Ex-4 treated mice, small islet-like cell clusters (ICCs) which all expressed TCF7L2 originated in the vicinity of the ductal epithelium. In isolated human exocrine cells *in vitro*, TCF7L2 overexpression increased proliferation of pancreatic ductal cells (3.69% Ki67 positive ductal cells in TCF7L2 overexpressing ductal cells, vs. 0.9% in GFP control, $p < 0.005$), together with induction of Pdx-1 and ngn3 expression by 4.2-fold and 5.1-fold respectively, compared to GFP-transfected ductal cells. In accordance what was seen in mouse models, ICCs next to ductal cells and insulin positive ductal cells were also present in patients with T2DM, but were rarely found in non-diabetic individuals.

Conclusion: Our finding implies a correlation of TCF7L2 expression and β -cell regeneration. TCF7L2 could trigger differentiation from ductal epithelial cells into β -cells *in vitro*. Our data support TCF7L2 as a new target for diabetes treatment to promote new β -cell formation.

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Liganded-thyroid hormone receptor and activin convert pancreatic AR42J-B13 cells into insulin-producing cells

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Background and aims: One goal of diabetic regenerative medicine is to instructively convert mature pancreatic exocrine cells into insulin-producing cells. It is still unclear which factors regulate pancreatic regeneration and β -cell neogenesis and which precursor cells are involved. Neurogenin 3 (ngn3) is a transcription factor that is essential for the differentiation of pancreatic endocrine cells. The expression of ngn3 is restricted to immature endocrine cells and is considered to be a marker of endocrine progenitor cells during development of the pancreas. Recently, we reported overexpression of liganded thyroid hormone receptor α (TR α) used by adenovirus vector (AdTR α) enhanced proliferation of pancreatic β -cells *in vivo*. In the present study, we investigated the influence of TR α on transdifferentiation of pancreatic exocrine cells to endocrine cells.

Materials and methods: Rat pancreatic AR42J-B13 cells possess exocrine and neuroendocrine properties. Previous reports indicated that in the presence of betacellulin or hepatocyte growth factor, activin-treated AR42J-B13 cells convert to insulin-producing cells. To identify the role of TR α in the process of differentiation of pancreatic β -cells, AR42J-B13 cells were infected with AdTR α with or without activin treatment. The expression of ngn3 or insulin mRNA were analyzed by quantitative RT-PCR. AdTR α -associated protein expression of ngn3 or insulin was analyzed by Western blot or immunocytochemistry, respectively. To explore whether liganded-TR α -induced transdifferentiation is direct or indirect effect, AdTR α -infected AR42J-B13 cells were concomitantly transfected with small interfering RNA (siRNA) against ngn3.

Results: Infection with AdTR α to activin-pretreated AR42J-B13 cells with T3-treatment enhanced the expression levels of ngn3 mRNA (2.6-fold) and protein (3.4-fold), compared without T3-treatment. No insululin-staining was observed in activin-pretreated AR42J-B13 cells. Overexpression of liganded-TR α induced the insulin protein expression in activin-treated AR42J-B13 cells. The siRNA-associated inhibition of reexpressed ngn3 significantly prevents AdTR α -induced transdifferentiation of AR42J-B13 cells into insulin-producing cells.

Conclusion: AR42J-B13 cells have the potential to differentiate into both exocrine and neuroendocrine cells. We have postulated that AR42J-B13 cells would provide a good *in vitro* model with which to study the differentiation of exocrine or neuroendocrine cells in islets. Present study supports the hypothesis that liganded-TR and activin induce ngn3 expression and endocrine differentiation of AR42J-B13 cells to insulin-producing cells. Liganded-TR α may be a target for therapeutic strategies that can induce the expansion and regeneration of pancreatic β -cells.

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Understanding the role of the transcription factor Math6 during endocrine cell development

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Background and aims: The bHLH transcription factor Neurogenin3 (Neurog3) initiates the endocrine differentiation program in the embryonic pancreas. We previously identified the bHLH factor Math6 as a component of the pancreatic transcriptional cascade downstream of Neurog3. Math6 is found in Neurog3+ endocrine progenitors and, mirroring Neurog3, is down-regulated in mature islet cells. While Math6 is essential for early embryonic development, the function of this factor during endocrine differentiation remains elusive. This work aims to gain insight into the molecular function of Math6 during the endocrine differentiation program initiated by Neurog3 in the pancreas.

Materials and methods: We have generated a recombinant adenovirus encoding a Math6-specific shRNA (shMath6) and used it to down-regulate expression of this factor in Neurog3-expressing pancreatic duct cells (mPAC). We have analyzed global changes in gene expression profiles in response to shMath6 using Affymetrix microarrays. To study cell cycle regulation, we have used cell count measurements, brdU incorporation, DNA content (propidium iodide), FACS sorting and phospho-histone3 staining.

Results: Math6 silencing affects significantly the expression of 293 genes in mPAC cells. Gene Ontology analysis has revealed cell cycle as the biological function most significantly represented among the modified genes. To establish the potential role of Math6 on cell cycle regulation, we have assessed the functional effects of shMath6 on cell proliferation and found that shMath6 decreases both cell number (-30%) and brdU incorporation (-47%). We have also analyzed cell cycle phase distribution and found that shMath6-treated cells accumulate at G2/M phases (+70%). This accumulation most likely occurs at the transition from G2 to M because phospho-histone3, a mitotic marker, is decreased in shMath6-expressing cells. We are currently determining the cell cycle genes that mediate these effects by validating potential candidates identified in the microarray analysis.

Conclusion: These results uncover a potential role of Math6 in the control of cell cycle progression and provide novel insights into the link between Neurog3 and the cell cycle, a poorly understood but important aspect of endocrine cell differentiation. These data may be useful for the development of *in vivo* protocols for the generation of replacement β cells for the treatment of diabetes.

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Pancreatic islet plasticity induced by sodium tungstate in IRS2-deficient mice modelJ. Oliveira^{1,2}, S. Rebuffat^{1,2}, A. García^{1,2}, A. Novials^{1,2}, R. Gomis^{1,2};¹IDIBAPS- Hospital Clinic/ Universitat de Barcelona, ²Ciberdem, Barcelona, Spain.

Background and aims: Insulin receptor substrate (IRS) 2 is one of the two major substrates for the insulin and insulin like growth factor signalling receptors and is required for various biological processes, such as nutrient metabolism, cell-cycle control, apoptosis and differentiation. Interestingly, IRS-2 is involved in the regulation of beta cell proliferation, as has been demonstrated using total IRS-2 knockout (IRS-2^{-/-}) mice. These animals develop type 2 diabetes associated with hepatic insulin resistance and a lack of compensatory beta cell hyperplasia. In this study we used sodium tungstate treatment as a tool to investigate the regulation of pancreatic islet plasticity in IRS-2^{-/-} mice. **Materials and methods:** 10 weeks wild type and knockout IRS2 C57Bl/6 mice were divided into two groups and given a solution of 2mg/ml of sodium tungstate in distilled water (treated group) or only distilled water (control group) for 21 days. During this period, physical status and blood glucose levels were recorded and glucose tolerance accessed before and after the treatment. Morphometric analysis of the pancreas was performed after the treatment. To determine differential gene expression between the four experimental groups we performed microarrays analysis (n=12) with isolated pancreatic islets.

Results: The administration of sodium tungstate significantly decreased glycemia in IRS-2^{-/-} mice while no changes were observed in the healthy animals (values day 0: 221±26 mg/dl versus values day 21: 134±23 mg/dl, n=9). By contrast, IRS-2^{-/-} control group developed hyperglycemia and diabetes during the experiment. Similarly, the administration of tungstate to IRS-2^{-/-} mice also improved glucose tolerance. Immunohistochemical analysis of insulin expression revealed, as expected, a reduced total beta cell area relative to total pancreas area in IRS-2^{-/-} control group (0.24%±0.2 versus 0.66%±0.2 in wild type animals, n=9). However, treated IRS-2^{-/-} mice showed an increment/preservation of pancreatic beta cell, reaching the levels found in the healthy treated animals (0.65%±0.3 versus 0.66%±0.2, n=7-9). In agreement with previous reports, tungstate treatment did not induce any significant modification in the healthy animals. IRS-2^{-/-} control animals had a higher number of apoptotic cells detected by cleaved caspase 3 immunostaining relative to total islet cell number than the healthy animals (24.7%±25 versus 0.37%±0.62). The treatment significantly decreased the rate of beta cell apoptosis in IRS-2^{-/-} mice (3.8%±2.4). Analysis of beta cell replication determined by the frequency of Ki67- positive beta cell nuclei revealed an increment of proliferation in treated IRS-2^{-/-} mice when compared to healthy animals (0.2%±0.2 versus 0.05±0.1). No Ki67- positive beta cells were found in IRS-2^{-/-} control animals. The analysis of the arrays from treated IRS-2^{-/-} animals identified 148 genes downregulated compared to IRS-2^{-/-} control group. Interestingly, 30% of these genes are the same genes that were upregulated in IRS-2^{-/-} control group and whose expression was restored by the tungstate treatment. Among these genes are the ones involved in inflammatory response and regulation of cell death.

Conclusion: Our results demonstrate that tungstate treatment reverses the diabetic phenotype of IRS-2 knockout mice and modifies the expression of a set of genes that seems to be participating in the recovery of endocrine function.

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The homeoprotein Alx3 interacts with Pax6 to regulate glucagon gene expression in pancreatic islet cells

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Background and aims: In previous studies we determined that Alx3, an aristaless-type homeodomain transcription factor, is expressed in pancreatic islets and participates in the regulation of insulin and somatostatin gene expression. In addition, we demonstrated that lack of Alx3 in mice leads to alterations in glucose homeostasis, including increased fasting blood glucose levels and impaired glucose tolerance tests. Insulin resistance associated to defective Akt-dependent signaling in liver and muscle develops with age and is evident in mice older than 36 weeks, whereas young Alx3-deficient mice exhibit mild hyperglycemia and normoinsulinemia accompanied by unal-

tered peripheral insulin sensitivity, thus suggesting a defect in glucose sensing mechanisms in islets. Gene expression studies carried out by quantitative RT-PCR revealed that islets from Alx3-deficient mice contain lower levels of glucagon mRNA that those obtained from control animals, suggesting that the glucagon gene is under regulation by Alx3. In the present study we aimed to determine the participation of Alx3 in the transcriptional regulation of the glucagon gene.

Materials and methods: Luciferase reporter genes were constructed by standard recombinant DNA techniques. Transfection studies were carried out in pancreatic islet-derived alfaTC1 cells, as well as in non-islet cells. Binding of Alx3 to promoter elements was investigated by chromatin immunoprecipitation and electrophoretic mobility shift assays.

Results: Chromatin immunoprecipitation from DNA extracted from isolated mouse islets and alfaTC1 cells confirmed that Alx3 occupies the glucagon gene promoter in vivo. The region of the glucagon promoter that recognizes Alx3 corresponds to the proximal segment spanning nucleotides -370 to +16, relative to the transcription initiation site. Transfections with an Alx3 expression vector and a luciferase reporter plasmid incorporating this region in Hela cells demonstrated that Alx3 transactivates the glucagon promoter. Electrophoretic mobility shift assays indicated that Alx3 binds specifically to the G1-54 and G1-52 sites of the G1 regulatory element, but not to the G1-50 site. The G1-54 site is known to be recognized by Pax6. Supershift, immunoprecipitation and GST-pull down assays indicated that Alx3 and Pax6 interact on this site. Transient transfection assays indicated that Alx3 and Pax6 enhance glucagon promoter activity synergistically.

Conclusion: Our results demonstrate a direct role of Alx3 in the transcriptional control of glucagon gene expression in pancreatic cells via heterodimeric interactions with Pax6.

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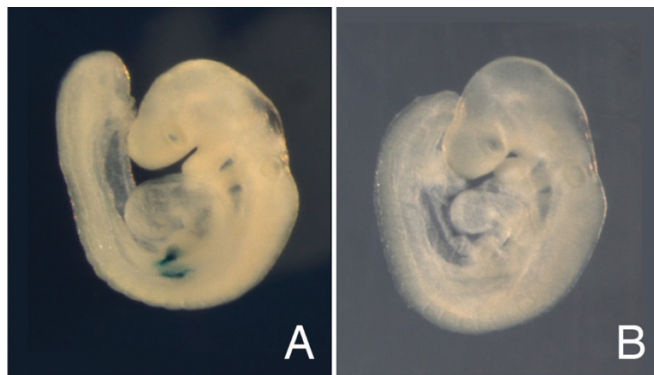
GATA4 is involved in pancreas specification via direct activation of Pdx1 in the endodermM. Carrasco^{1,2}, I. Delgado¹, B. Soria^{1,2}, F. Martín^{1,2}, A. Rojas^{1,2};¹Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), ²CIBER de Diabetes y Enfermedades Metabólicas (CIBERDEM), Sevilla, Spain.

Background and aims: The GATA family of zinc finger transcription factors have been shown to be involved in the specification and differentiation of the endoderm and its derivatives. GATA4 is the first member to be expressed in the mouse embryo. Inactivation of GATA4 in the germ line leads to embryonic lethality and Gata4 null embryos display defects in heart and endoderm morphogenesis. Lack of GATA4 impairs pancreas induction and the cells in the foregut do not express Pdx1, the earliest pancreatic marker. These results suggest that GATA4 is crucial for pancreas formation, although the mechanisms involved are not well established. We aim to establish a functional interaction between GATA4 and Pdx1 for pancreas induction.

Materials and methods: We are using in vitro and in vivo approaches to study the transcriptional regulation of Pdx1 by GATA4 transcription factors. We are using EMSA, ChIP, transfection analyses and transgenic mice harboring the Pdx1 promoter controlling the lacZ reporter gene.

Results: A 5Kb promoter region of the Pdx1 gene has been previously shown to recapitulate the embryonic expression from earliest stages of development. This promoter region contains several conserved GATA binding sites. We show that these GATA sites are able to bind GATA4 recombinant protein in vitro. We also show that the GATA sites in the PDX1 promoter are occupied by GATA4 in mPAC pancreatic cell line. Moreover, mutations of the GATA sites negatively affect the activation of a reporter construct containing the luciferase under the control of Pdx1 promoter region in transfection assays. Finally, our analyses in transgenic mice demonstrate the requirement of the GATA sites for the initial activation of Pdx1 in the foregut.

Conclusion: Our results suggest that GATA4 is involved in pancreas specification by directly activating the transcription of Pdx1 in the pre-pancreatic endoderm.



Conserved GATA sites in the Pdx1 promoter are required for the initial transcriptional activation in the foregut. Representative X-gal stained transgenic embryos harboring the wild type (A) or mutated GATA sites (B) of the Pdx1 promoter fused to lacZ reporter gene are shown.

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Endothelial factors increase islet-cell proliferation in adult mouse and human pancreatic islets

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Background and aims: One promising approach for the cure of diabetes is beta cell replacement and regeneration therapies. The short supply in beta cells from cadaveric donors and the low replication capacity of beta cells have stimulated efforts to find ways of re-generating beta cells *in vitro* and *in vivo*. One of the non-transcription factors that controls proliferation and differentiation of pancreatic stem/progenitor cells is the vascular endothelial and its angiogenic factors. In this regard, the studies raise the possibility that endothelial cells and its angiogenic factors create a permissive environment that helps proliferation and differentiation of pancreatic stem/progenitor cells. The aim of this study is to identify the angiogenic signals involved in proliferation of adult islet-cells, to characterize these signals and to study the molecular mechanisms of their action.

Materials and methods: In vitro experiments: mouse and human islets were cultured 48h in 1% hypoxia or in the presence of VEGFA, anti-VEGFR1 and both agents. In vivo experiments: mice were injected with PBS or VEGFA plus anti-VEGFR1. VEGFA, VEGFR1 and VEGFR2 mRNA expression was measured by RT-PCR. VEGFR1 and VEGFR2 protein presence was evaluated by immunofluorescence. Islet-cell proliferation was measured by BrdU incorporation and Ki67. Analysis of replicating cells were performed by double immunofluorescence (BrdU+Insulin, Ki67+Insulin, Ki67+CD31+Insulin and Ki67+Pdx1+Insulin). VEGFA production, insulin content and glucose-induced insulin release was assayed by ELISA. Akt expression and Akt phosphorylation (serine 473) were measured by Western blot.

Results: Pancreatic islets had a higher VEGFA, VEGFR1 and VEGFR2 expression than aortic tissue. Beta cells were positive for VEGFR1 and VEGFR2. 1% hypoxia culture increased VEGFA, VEGFR1 and VEGFR2 expression. In vitro culture in the presence of VEGFA plus anti-VEGFR1 induced: i) a significant increase ($p < 0,05$) in VEGFA, VEGFR1 and VEGFR2 expression; ii) a significant increase ($p < 0,01$) in islet-cell proliferation and iii) a significant increase ($p < 0,001$) in glucose sensitivity and glucose-induced insulin secretion. Proliferating cells were positive for insulin and Pdx1 and negative for CD31. In vivo administration of VEGFA plus anti-VEGFR1 induced: i) a significant increase ($p < 0,01$) in beta cell proliferation and ii) a significant increase ($p < 0,001$) in glucose sensitivity and glucose-induced insulin secretion. Akt phosphorylation (serine 473) significantly increased ($p < 0,05$) in the presence of VEGFA plus anti-VEGFR1.

Conclusion: Adult beta cells can successfully proliferate, *in vivo* and *in vitro*, in the presence of angiogenic factors, without losing their functionality. Probably, these effects are mediated via Akt signalling pathways.

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Wnt4 is a novel regulator of pancreatic beta cell proliferation and upregulated in response to exendin-4

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Background and aims: The Wnt-signaling pathway regulates beta-cell functions. It is not known how the expression of endogenous Wnt-signaling molecules is regulated in beta-cells. Therefore, we investigated the effect of antidiabetic drugs and glucose on the expression of Wnt-signaling molecules in beta-cells.

Materials and methods: Preparation of primary islets. Analyses of Wnt and TNFalpha molecules by semiquantitative PCR and western blotting. Transient transfections and proliferation assays of INS-1 beta-cells ($[^3\text{H}]$ -thymidine uptake). Quantification of insulin secretion. Knock-down (siRNA) of Wnt4 in beta-cells.

Results: Exendin-4 significantly increased the expression of Wnt4 in beta-cells on the mRNA level (2.8-fold) and the protein level (3-fold) ($p < 0.001$). The effect was dose-dependent with strongest stimulation at 10 nM and it was maintained after long term stimulation over four weeks. Addition of exd-(9-39), a GLP-1 receptor antagonist, abolished the effect of exendin-4. Treatment with glucose, insulin or other antidiabetic drugs had no effect on the Wnt4 expression. The expression of other Wnt-signaling components was not regulated by any of the stimuli. Functionally, Wnt4 antagonized the activation of canonical Wnt-signaling in beta-cells. Wnt4 had no effect on glucose-stimulated insulin secretion and insulin gene expression. Knocking down Wnt4 decreased beta-cell proliferation to 45% of controls ($p < 0.05$). In addition, Wnt4 and Exendin-4 treatment decreased the expression of TNFalpha mRNA in primary beta-cells.

Conclusion: These data demonstrate that stimulation with exendin-4 increases the expression of Wnt4 in beta-cells. Wnt4 modulates canonical Wnt-signaling and acts as regulator of beta-cell proliferation and inflammatory cytokine release. This suggests a novel mechanism through which GLP-1 can regulate beta-cell proliferation

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Down regulation of sFRP5 in pancreatic islets promotes beta cells proliferation in obesity

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Background and aims: In obesity, beta cell compensates for insulin resistance through increased beta cell mass and function in order to maintain glucose homeostasis. Replication, neogenesis or apoptosis are the main mechanisms involved in beta cell plasticity and are essential in avoiding the onset of hyperglycemia. Therefore, mechanisms regulating this process must be fully characterized. There is growing evidence suggesting that expanding adipose tissue that occurs in obesity affects both the mass and function of beta cells. Data shows that the adipose tissue that surrounds the pancreas is able to specifically secrete some proliferative signals which are partially responsible for this increase in beta cell mass. The objective of this study is to investigate gene expression changes induced by adipose tissue in islets during the progression towards obesity and explore the involved signaling pathway using a diet-induced-obesity model, the Cafeteria rat.

Material and methods: Wistar rats were fed with either standard (STD) chow or a cafeteria (CAF) diet for 10 and 30 days. At the end of the treatment, pancreatic islets were isolated. Gene expression profiles were realized by Affymetrix GeneChip® Rat Genome 230 2.0 Array (n=10). Normalization and differential expression analysis were performed with RMA and LIMMA software. Gene and protein expression were carried out. Proliferation, cellular death and insulin secretion have been measured in INS1E cell line and dissociated islets transfected by sFRP5 siRNA. Protein signaling pathways were studied in isolated islets from STD and CAF rats using Panorama cell signaling antibodies arrays and the most relevant results confirmed by Western Blot analysis.

Results: After statistical analysis, some genes (n=6) were differentially expressed between CAF vs STD islets. Interestingly, one of these genes was sFRP5 (Secreted frizzled-related protein 5), negative regulator of the Wnt signaling pathway involved in developmental processes, including proliferation, growth control and cell fate determination. Microarrays analysis revealed a decrease in SFRP5 gene expression in CAF islets (Fold Change: 2.30;

$p < 0.05$). This reduction was confirmed by quantitative real time PCR (FC: 2.08; $p < 0.05$) and by Western Blot. To validate our results obtained in our animal model, sFRP5 down regulation was performed using siRNA transfection in INS1E cells and in dissociated rat islets. The decrease of sFRP5 expression was confirmed by RT PCR (INS1E: -81.4 %, islets: -76.0 %; $p < 0.05$), western blot and immunofluorescence analysis. Using BrdU incorporation we observed that this down-regulation promotes an increase of proliferation (INS1E: +51.1 %, islets: +40.2 %; $p < 0.05$), without changes in apoptosis measured by flow cytometric analysis (INS1E: $18.1\% \pm 2.76$, Control siRNA INS1E: $18.4\% \pm 1.41$, sFRP5 siRNA INS1E: $19.8\% \pm 1.98$) confirming *in vivo* results. The first studies suggest that insulin secretion was not affected. The analysis of protein arrays identified several up-regulated proteins involved in the progression of cell cycle and also proteins belonging to cell signaling pathways such as MAPK, JNK.

Conclusion: Results presented indicate that, in obesity, pancreatic islets sFRP5 down-regulation is involved in beta cell proliferation. This key finding will pave the way for understanding the mechanisms that regulate beta cell mass compensation for obesity.

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Human mesenchymal stem cells modulate dendritic cells presentation of islet antigen GAD65 in type 1 diabetes

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Background and aims: Mesenchymal stem cells (MSCs) exert an immunosuppressive effect on virtually any component of the immune system, including suppression of monocyte differentiation and maturation into dendritic cells (DC). We recently demonstrated that human MSC abrogated *in vitro* a pro-inflammatory Th1 response to islet antigen GAD in type 1 diabetes, impairing IFN- γ and inducing anti-inflammatory IL-4 production. In the present study, we evaluated whether human MSCs have, *in vitro*, an immunomodulatory potential directly on DC, modulating the balance of Treg cells subset. **Materials and methods:** Human bone marrow-derived MSCs were isolated and characterised. Peripheral blood mononuclear cells (PBMCs) were obtained from 7 type 1 diabetic patients at disease onset. Monocyte derived dendritic cells (DC) were differentiated from CD14⁺ cells in the presence of GM-CSF and IL-4, then pulsed with GAD65, matured in the presence of TNF- α and IL-1 β and cultured with MSCs for 24-48 hours (optimal ratio 10:1). The expression of CD1a, CD14 as well as maturation/activation markers on DCs (CD83, B7H1, B7H2) were evaluated by flow cytometric analyses. Then DCs cultured or not in the presence of MSCs were subsequently cocultured with autologous CD14⁺ lymphocytes. After 5 days coculture, IFN- γ ELISpot responses of recovered lymphocytes were assayed. Moreover, levels of prostaglandin E₂ (PGE₂), IFN- γ , IL-6, IL-10, IL-17 and TGF- β in supernatants collected after 24-48h +/-MSCs/DCs culture and after 5 days T lymphocytes/DCs coculture were measured by ELISA. Furthermore, the induction of T regulatory (CD4/CD25 high FOXP3⁺) cells subset were evaluated by flow cytometric analyses.

Results: Four diabetic patients showed a positive IFN- γ T cell response to GAD65 (mean spots 40 ± 26). Pre-incubation of the respective GAD65-pulsed DC with MSCs, inhibited the T cell activation, resulting in a significant decrease in the number of IFN- γ spots (mean spots 22 ± 18). Cocultures with MSCs did not affect the expression of CD83, B7H1, B7H2 on DCs, while significantly decreased secretion of IFN- γ and IL-6 and increased secretion of IL-10, PGE₂ and TGF- β were observed in supernatants collected. Moreover, MSCs cocultures induced regulatory T cells.

Conclusion: Human MSCs impair islet GAD antigen presentation to autoreactive T lymphocytes and significantly modifies the cytokine secretion profile of DC and T cell subsets, promoting an anti-inflammatory environment; even if do not inhibit DC maturation.

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The role of B1 cells in the early steps of type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) is generally known as a T-cell mediated autoimmune disease where the pancreatic beta-cells are destroyed and insulin secretion abrogated with severe metabolic consequences. Yet, B lymphocytes have been proven necessary for pathogenesis and detection of autoantibodies (autoAbs) to beta-cell antigens is one of the earliest indicators of disease. In particular, insulin autoAbs have predictive value both in human patients and in the non-obese-diabetic (NOD) mouse, a widely used animal model that spontaneously develops T1D in a similar manner to the human condition. Nevertheless, the origin and the role of beta-cell specific autoAbs in T1D development remain obscure. Unraveling the root of autoAbs generation and identifying linked B cell dysfunctions leading to disease will allow optimal diagnosis and early therapeutic interventions in T1D. B1 lymphocytes constitute a distinct B cell population of fetal origin, characterized by the expression of CD5 molecule and secretion of Natural antibodies (NAbs). We put forward the unexplored hypothesis that B1 cells and the natural autoantibodies they produce, are involved in T1D early pathogenesis by modulating the autoAbs repertoire.

Materials and methods: We have analyzed the phenotype and function of B1 cells in several organs of the NOD mouse in comparison to the C57BL/6 con-

tol mouse strain. Thus we have characterized by flow cytometry the distribution of B1 cells in different organs as well as the expression of molecules on their cell surface. Also, we have isolated B1 cells and determined by ELISPOT their *ex vivo* ability to secrete antibodies recognizing T1D related autoantigens. In addition we have characterized by Real Time PCR the expression of the innate Toll-like Receptors (TLRs) and performed *in vitro* cell cultures of purified B1 cells with and without agonist to TLR4 (LPS) and TLR9 (CPG) to characterize their response to innate stimuli. Proliferation was measured through thymidine incorporation and ELISA and ELISPOT techniques were used to analyze the secretion pattern of the cytokine IL10 and of autoAbs in these cell cultures.

Results: We have observed that B1 cells from the peritoneal cavity are the main secretors of IgM recognizing T1D related autoantigens. Further, young NOD mice without pancreatic infiltration presented a B1 cell repertoire with increased self-reactive in comparison to C57BL/6 mice. Also NOD B1 cells showed a higher expression of activation markers prior to pathogenesis onset indicating an increased basal level of activation in these cells. Interestingly, the levels of expression of several TLR were increased in NOD B1 cells and *in vitro* stimulation with TLR agonist to TLR4 and TLR9 resulted in the secretion of higher amounts of IgM recognizing T1D related antigens and less IL10 secretion by NOD B1 cells in comparison to the control mouse strain.

Conclusion: NOD B1 cells present an antibody repertoire with increase self reactivity and are more prone to secrete autoantibodies upon innate stimulation. We hypothesize that B1 cells may contribute to T1D onset and will further explore how innate stimuli may determine the reactivity of the NOD B1 cell repertoire and contribute to the development of T1D.

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Toll like-receptor 4: regulator of energy metabolism in the non-obese diabetic mouse

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Background and aims: Type 1 diabetes results from the immune-mediated destruction of autologous insulin-producing beta cells. Recent studies implicate that mechanisms controlling energy metabolism could be involved in the progression of beta cell destruction and disease development. Toll-like receptor 4 (TLR4) not only serves as receptor for bacterial lipopolysaccharides on innate immune cells, but also for metabolites such as fatty acids. Aside from its involvement in the pathogenesis of autoimmune diseases, TLR4 could therefore contribute to metabolic abnormalities in pre-diabetic states. We hypothesized that TLR4 determines a diabetes-modulating metabolic phenotype in NOD mice. To assess the potential impact of TLR4 during the pre-diabetic state, we performed comprehensive metabolic phenotyping of a TLR4-expressing ($T4^{+/+}$) and a TLR4-deficient ($T4^{-/-}$) substrain of the non-obese diabetic (NOD) mouse.

Materials and methods: Body weight and food intake of female normoglycemic TLR4-expressing and TLR4-deficient NOD mice were monitored from 7 to 22 weeks of age. Metabolic phenotyping was performed during three 12-hours light- and dark-cycles in a modular calorimetric system to determine physical activity, food- and water-intake as well as respiratory activity. Glucose metabolism was determined in fasted mice with intraperitoneal glucose tolerance tests (IPGTT).

Results: Compared with $T4^{+/+}$ mice, $T4^{-/-}$ mice show accelerated body weight gain, which occurs independently of food intake. $T4^{+/+}$ and $T4^{-/-}$ mice have a comparable energy expenditure during both light- ($T4^{+/+}$: 15.5 ± 0.7 kcal/(h x kg); $T4^{-/-}$: 14.4 ± 0.7 kcal/(h x kg)) and dark-cycles ($T4^{+/+}$: 19.2 ± 1.3 kcal/(h x kg); $T4^{-/-}$: 17.4 ± 1.1 kcal/(h x kg)). Physical activity as assessed by the distance covered is higher ($p < 0.01$) in dark- than in light-cycles, but is not different between both groups during light- ($T4^{+/+}$: 113 ± 7 m, $T4^{-/-}$: 137 ± 8 m) and dark-cycles ($T4^{+/+}$: 290 ± 8 m, $T4^{-/-}$: 262 ± 178 m). However, $T4^{-/-}$ mice exhibit lower respiratory exchange rates (RER , VCO_2/VO_2) in both light- ($T4^{+/+}$: 0.95 ± 0.03 , $T4^{-/-}$: 0.85 ± 0.03 , $p < 0.05$) and dark-cycles ($T4^{+/+}$: 1.00 ± 0.03 , $T4^{-/-}$: 0.90 ± 0.03 , $p < 0.001$). Moreover, $T4^{-/-}$ mice have markedly lower glucose tolerance as assessed from the incremental area under glucose curve of the IPGTT (587.6 ± 152.4 mmol/h) than $T4^{+/+}$ mice (203.9 ± 102.8 mmol/h, $p < 0.05$).

Conclusion: Selective TLR4 deficiency impairs glucose tolerance in nondiabetic NOD mice which may relate to accelerated fat oxidation and accelerated body weight gain despite no effect on physical activity and food intake. These findings suggest that TLR4 contributes to the progression of insulin-deficient diabetes in NOD mice by affecting the energy metabolism.

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Identification of a monomorphic humoral epitope in the ZnT8 C-terminal domain

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Background and aims: ZnT8 is a major target in human type 1 diabetes (T1D). The identification of key humoral epitopes in this autoantigen may provide clues to the etiology of the disease, and will increase the range of available biomarkers. Our initial studies demonstrated that the majority of ZnT8-reactive sera recognize the final 100aa of the molecule, and that residue 325 is a major determinant in 2 epitopes linked to a common genetic polymorphism. The goal of the current study was to identify non-polymorphic epitopes in ZnT8 that are recognized by “un-restricted” sera.

Materials and methods: The C-terminal domains of human and mouse ZnT8 are ~80% identical. However, the mouse probe is not precipitated by the majority of human T1D sera. Thus to identify key residues we systematically “humanized” the mouse probe, either by creating chimeras, or site directed mutagenesis of variant residues, and evaluated the probes in radioimmunoassays.

Results: Analysis of chimeric and truncated probes suggested that a major non-polymorphic epitope was dependent on residues located between aa 320 and aa 360, which contains 11 variant residues including the polymorphic aa 325. No single substitution gave significant restoration of binding to “un-restricted” human sera. However, when clusters of structurally adjacent variant residues were also changed a region of antigenicity was revealed that depended on residues R332, E333, K336 and K340. Using 112 sera from newly diabetic subjects tested with the human aa325Q and m-R₃₂₅R₃₃₂E₃₃₃K₃₃₆K₃₄₀ probes, 39.3% of the subjects were ZnT8(Q)A+, of which 38.6% (17/44) also recognized the mouse probe.

Conclusion: We conclude that the mRREKK probe identifies a major non-polymorphic epitope that may add further to the diagnostic utility of ZnT8 autoantibodies.

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CXCR3+, CCR4+ and CD25hi T memory cells and cytokines levels: an analysis during the initial phase of type 1 diabetes

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Background and aims: Previous studies have reported an important role of the chemokine receptors CXCR3 and CCR4, which are associated with Th1 and Th2 T memory cell subsets respectively and involved in their extravasations into inflamed pancreatic islets, together with changes in T regulatory (T reg) subset, in the initial phase of Type 1 diabetes (T1D). However, the significance of the changes in CXCR3+ (Th1 associated), CCR4+ (Th2 associated) and CD25hi subsets (T reg associated) of the T memory cells as well as in chemokine/cytokine levels, interferon- γ inducible chemokine (IP-10) (Th1 associated), thymus- and activation-regulated chemokine (TARC) (Th2 associated) and transforming growth factor β (TGF β) (Treg associated), in recent onset T1D, have not yet been clarified. Therefore, the aim of this study was to analyze (a) the percentage of CXCR3+, CCR4+, CD25hi subsets of T memory cells and (b) chemokine/cytokine levels IP-10, TARC and TGF β , in peripheral blood in 26 recent-onset T1D patients in insulin-requiring state (IRS) at the onset (group A), 12 T1D patients in the state of clinical remission (CR) (group B), as well as in 20 healthy, age-matched control subjects (group C).

Materials and methods: T1D was diagnosed in accordance to WHO criteria. The CR was defined as optimal metabolic control without insulin lasting >30 days. The percentages of CXCR3+, CCR4+ and CD25hi T memory cell subsets were analyzed in peripheral blood by using four-color immunofluorescence staining and flowcytometry. IP-10, TARC and TGF β were determined by ELISA.

Results: We found that there was no difference among the groups concerning the percentage of T memory cells (A: 27.33 ± 8.52 vs B: 24.15 ± 6.52 vs C: 27.63 ± 6.52 %, A vs B vs C, $p = \text{NS}$). However, when the percentage of CXCR3+ T memory cells was analyzed, we found that in groups A and B it was significantly lower than in group C (A: 40.15 ± 11.55 ; B: 42.13 ± 11.11 ; C: 53.12 ± 6.34 %; A vs C: $p < 0.001$; B vs C: $p < 0.01$), while there was no differ-

ence between groups A and B. Simultaneously, the percentage of CCR4+ and CD25hi T memory cells was also found to be significantly lower in groups A and B than in group C (A: 31.59 ± 9.80 ; 0.23 ± 0.03 , B: 31.42 ± 8.13 ; 0.23 ± 0.10 , C: 40.92 ± 7.20 ; $0.26 \pm 0.06\%$, respectively; A vs C and B vs C, $p < 0.05$), again without difference between groups A and B. On the other hand, IP-10 and TARC levels were significantly higher in groups A and B than in group C (A: 141.99 ± 69.59 ; 398.14 ± 333.09 , B: 133.03 ± 63.19 ; 387.06 ± 124.57 , C: 85.24 ± 19.82 ; 236.88 ± 89.19 pg/ml, respectively; A vs C and B vs C, $p < 0.05$), while TGF β levels were significantly lower in groups A and B than in group C (A: 4784.37 ± 1858.41 B: 5243 ± 1228.95 C: 10690.50 ± 7246.65 pg/ml; A vs C and B vs C, $p < 0.05$), also without differences between groups A and B.

Conclusion: Our results shown that the onset of T1D was associated with the decrease in CXCR3+, CCR4+, CD25hi subsets of T memory cells and TGF β levels, together with increase in IP-10 and TARC, which persisted during the clinical remission, presumably reflecting both Th1 and Th2 extravasation into pancreatic tissue and impairments in immunoregulation. The results imply that the onset of the disease could be modified on the level of these subsets of T memory cells and associated cytokines.

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Coxsackievirus up-regulates IL-17 immunity in human type 1 diabetes
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Background and aims: Coxsackievirus infections have been associated with beta-cell autoimmunity. In acute viral myocarditis, up-regulation of IL-17 correlated positively with the replication of Coxsackievirus B3 due to the interference with the induction of Th1 immunity and virus eradication. Our aim was to study the activation of coxsackievirus induced IL-17 and IFN- γ responses in human type 1 diabetes (T1D).

Materials and methods: We studied the gene expression levels of IL-17 and IFN- γ in Coxsackievirus B4 strain (CVB4) -stimulated peripheral blood mononuclear cells (PBMCs) from 14 children with T1D and from 13 healthy children with HLA risk genotype for T1D and from 14 children without a risk genotype. PBMCs were stimulated for 7d with heat inactivated CVB4 and cells were collected for gene expression analysis of IL-17 and IFN- γ with qRT-PCR. In addition, we studied the intracellular expression of cytokines supporting IL-17 immunity, namely IL-1 β (IL-1 β) and IL-6 in myeloid dendritic cells (mDCs) and monocytes in Coxsackievirus B5 (CVB5) -stimulated PBMCs from three healthy individuals.

Results: The IFN- γ /IL-17 ratio in CVB4 stimulated PBMCs differed between the study groups ($p = 0.03$ in Kruskal-Wallis test). Healthy children without genetic risk for T1D showed higher IFN- γ /IL-17 ratio than children with T1D (median values 80.2 vs. 24.6, $p = 0.02$ in Mann-Whitney U-test). A similar trend for higher IFN- γ /IL-17 ratio was found in healthy children without genetic risk when compared to healthy children with genetic risk (median values 80.2 vs. 11.9, $p = 0.08$). The healthy children with genetic risk for T1D and children with T1D did not show difference in IFN- γ /IL-17 ratio (median values 11.9 vs. 24.8, $p = 0.8$). In vitro stimulation with Coxsackievirus up-regulated IL-1 β and IL-6 in mDCs and monocytes.

Conclusions: We conclude that coxsackievirus induced IL-17 expression is increased in relation to IFN- γ in children with T1D. This may impair virus eradication mechanisms and lead to invasive, chronic or recurrent coxsackievirus infections in patients with T1D. Based on our previous data of detrimental effect of IL-17 on human islets and mouse beta-cells, the up-regulation of IL-17 immunity by CVB may also increase beta cell death and the release of beta-cell derived antigens in the pancreatic islets.

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Adipocytes of non-obese diabetic mice exhibit enhanced toll-like receptor 4-mediated proinflammatory activity

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Background and aims: Recent studies on the development of type 1 diabetes implicate a role of the adipose tissue mass in disease progression. Adipocytes, the major cellular component of the adipose tissue, had been identified as important source of mediators promoting the progression of inflammatory processes. As the release of inflammatory mediators from adipocytes is under strict control of the Toll-like receptor 4 (TLR4), we hypothesized that a genetic background, predisposing for diabetes, affects the TLR4-dependent reactivity of adipocytes thereby contributing to diabetes progression. We therefore performed comparative analyses of the TLR4 reactivity of adipocytes from mouse strains without diabetes risk (C57BL) or with a risk to develop insulin-deficiency diabetes (non-obese diabetic (NOD) mouse) or the metabolic syndrome (New Zealand obese (NZO) mice).

Materials and methods: Adipocytes were isolated from the visceral fat depot of C57BL-, NOD and NZO mice. TLR4-dependent stimulation of preadipocytes and in vitro matured adipocytes was assessed by incubation of the cells with the TLR4 ligand lipopolysaccharide (LPS, 24h). The release of the proinflammatory mediators monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) was quantified by ELISA.

Results: After LPS exposure (100 ng/ml) (pre-)adipocytes of TLR4-expressing C57BL-mice release 7 - 8-fold higher concentrations of IL-6 and MCP-1 than cells of TLR4-deficient animals (< 0.2 ng/ml IL-6 and MCP-1) ($p < 0.01$), thus proving the TLR4-dependence of adipocyte reactivity. The release of proinflammatory mediators from (pre-)adipocytes of different mouse strains in response to LPS was determined. Unstimulated preadipocytes of NOD-mice released higher IL-6- (5.8 ± 2.1 ng/ml) and MCP-1-amounts (22.1 ± 8.3 ng/ml) than cells of C57BL- (2.4 ± 1.9 ng/ml IL-6; 11.4 ± 0.7 ng/ml MCP-1; $p < 0.05$) and of NZO-mice (0.7 ± 0.8 ng/ml IL-6 ($p < 0.001$); 20.1 ± 10.1 ng/ml MCP-1). LPS-exposed preadipocytes of NOD-mice released increased IL-6- (32.1 ± 9.1) and MCP-1-levels (78.9 ± 10.0 ng/ml) when compared to cells of C57BL- (15.0 ± 7.2 ng/ml IL-6; 56.9 ± 17.7 ng/ml MCP-1; $p < 0.05$) and NZO-mice (10.3 ± 7.7 ng/ml IL-6; 37.7 ± 16.2 ng/ml MCP-1; $p < 0.01$). Interestingly, unstimulated mature adipocytes of NOD- and C57BL-mice released similar levels of IL-6 ($6.2 - 6.5$ ng/ml) and MCP-1 ($20.8 - 27.1$ ng/ml) which were significantly higher than the levels released from NZO-mouse-derived cells (0.9 ± 0.8 ng/ml IL-6; 13.3 ± 8.4 ng/ml MCP-1; $p < 0.05$). LPS-stimulated mature adipocytes of NOD- and C57BL-mice released higher IL-6- ($19.9 - 23.3$ ng/ml) and MCP-1-levels ($75.7 - 84.9$ ng/ml) than cells of NZO-mice (7.7 ± 4.7 ng/ml IL-6; 35.8 ± 16.2 ng/ml MCP-1; $p < 0.01$).

Conclusion: Our findings demonstrate that the genetic background, which determines the risk to develop a disturbed glucose metabolism, affects the TLR4-responsiveness of adipocytes. Whereas NZO mouse-derived (pre-) adipocytes exhibit a reduced reactivity, the genetic background of the NOD mouse which predisposes for the development of insulin deficiency diabetes, is associated with an increased TLR4-dependent proinflammatory reactivity of preadipocytes.

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Correlation of inflammatory response gene expression in blood mononuclear cells to islet infiltration stages in LEW.1AR1-iddm rat model of autoimmune diabetes

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Background and aims: The LEW.1AR1-iddm rat is an animal model of spontaneous autoimmune diabetes. Islet infiltration occurs within a narrow time range between day 40 and 50 after birth resulting in progressive beta cell destruction and overt diabetes around day 60. It was the aim of this study to correlate the gene expression profile in mononuclear cell of the blood (PBMC) with islet infiltration and beta cell mass destruction at different stages of islet infiltration.

Materials and methods: LEW.1AR1-iddm rats were killed at day 40, 45, 50, 55 and 60 ($n = 4 - 8$ per time point) with subsequent sampling of organs. RNA was purified from isolated mononuclear blood cells and pancreas draining lymph nodes. Gene expression was quantified for proinflammatory cytokines (TNF α , IFN γ , IL-1 β), antiinflammatory cytokines (IL-4, IL-10), T cell

marker (CD25, CTLA-4/CD152, Neuropilin), L-Selectin, TGF- β and FoxP3 by RT-PCR analyses using specific oligonucleotide probes. Furthermore RT-PCR Array analyses (Rat Inflammatory Response and Autoimmunity, Qiagen) covering chemokine panels were performed with blood RNA samples at the early stage of islet infiltration. Serial pancreatic sections were stained for Haematoxylin-Eosin (HE), immune cells and insulin to document infiltration state and level of beta cell destruction.

Results: At the stage of organ preparation all rats were normoglycaemic with blood glucose levels in the range between 5 and 7.5 mmol/l. 34 % (18/52) of the rats showed infiltrated islets ranging from 27 % (day 40) to 50 % (day 60). At day 40 gene expression of TNF α , IFN γ , IL-4, IL-10, CTLA-4/CD152, Neuropilin, L-Selectin, TGF- β and FoxP3 was significantly ($p < 0.05$) higher in PBMC from rats with islet infiltration compared to rats without signs of insulinitis. However at this early time point of islet infiltration the expression levels of these markers were not increased in pancreatic draining lymph nodes. Gene expression of PBMC showed that pancreatic infiltration is accompanied with high expression levels of chemokines, chemokine receptors (Ccl17, Ccl3, Ccl6, Ccr1) and regulatory genes (Cebpb, Ripk2, Nfkb1). Infiltrated islets at day 40 showed minimal loss of beta cells in the range of 10–20 % but a drastic reduction of insulin immunostaining (Insulin/Dapi: 3.8 ± 0.4 vs. 0.6 ± 0.1). At day 50 the expression levels of all cytokine/T cell markers significantly decreased and did not show significant differences between cohorts with infiltrated and normal islets. At day 55 CTLA-4/CD152, CD25 and FoxP3 expression significantly increased in PBMCs from infiltrated islets with progressive insulinitis and loss of insulin producing cells. At day 60 the expression profile was characterized by expression of the proinflammatory cytokines TNF α , IFN γ and IL-10.

Conclusion: The data indicate that in PBMCs the gene expression of proinflammatory cytokines and chemokines are significantly increased at the early stage of islet infiltration around day 40. The expression state of cytokines and T-cell activation then follows a two-peak model with increases at early islet infiltration, a nadir during progressive T-cell infiltration and a second peak at day 55 and 60 when beta cell destruction is > 70 %. Thus, gene expression profiles of chemokines/cytokines in PBMCs may serve as surrogate markers for different stages of islet inflammation in type 1 diabetes.

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Conclusion: The findings show that the C terminus of IL-7 is important for signal transduction and i.e. identifying candidate residues to target for obtaining an IL-7 antagonist with therapeutic potential.

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Controlling autoimmunity and transplant rejection by targeting interleukin-7 mediated signalling

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Background and aims: Type 1 diabetes mellitus is an autoimmune disease characterized by the destruction of islet beta cells by autoreactive T cells. We have reported that increased concentrations of Interleukin-7 (IL-7) can lead to an expansion of memory T cells, including autoreactive T cell clones, leading to reduced graft survival. Thus IL-7 antagonists could be useful drugs to modulate autoimmunity. The aim of this work is to identify mutants of IL-7 that could act as antagonists.

Materials and methods: The IL-7 structure and hypothetical binding of IL-7 to the IL-7R complex and in particular the common gamma chain was used to identify candidate amino acids for mutants. The wild type and glycine substituted mutant IL-7 proteins were expressed in E coli, and purified from inclusion bodies with subsequent refolding. The biological activity of the mutants was analyzed by measuring STAT5 phosphorylation in CD4 $^{+}$ T cells after IL-7 treatment by FACS. Binding to the IL-7Ralpha chain was measured with a competition assay, in which biotinylated wild type IL-7 competes with the mutant protein for binding to immobilized IL-7Ralpha.

Results: Sixteen residues in the D helix of IL-7 were identified as candidate targets for mutation on the basis of their hypothesized interaction with the common gamma chain and their evolutionary conserved nature. Thus, far, 6 of 16 mutants, with and without His-tag, have been prepared and tested for STAT5 phosphorylation. Mutation of residue W142 to glycine leads to a complete loss of pSTAT5 signaling capacity (7% of wild type activity). Markedly decreased activity was observed for mutants I138G (16%), N143G (19%) and K144G (24%), while the mutants R133G (76%) and E137G (93%) showed activity in the range of wild type protein. His-tagging did not interfere with the biological activity facilitating further complete mutational studies at the relevant residues. In a competition assay it was shown that the W142G mutant bound to the IL-7Ralpha chain with low affinity and could displace wild type IL-7 if present in high concentration.

PS 019 Transplantation

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Histological examination of human islet transplants

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Background and aims: In Type 1 diabetes (T1D) the immune system destroys the beta cells. Islet transplantation is still experimental as only a relatively small percentage of patients remain insulin independent after two years. However, islet transplantation offers the potential to be a cure for T1D. The aim of this project was to determine whether protocol liver biopsies after islet transplantation would yield islets for histological analysis and to determine the cause of loss of islet function.

Materials and methods: Patients who received an islet transplant for severe hypoglycaemic unawareness had a liver biopsy performed at the time of the second and subsequent transplant. Every seventh section of the biopsies was stained with insulin and counterstained with H&E to identify the islets.

Results: Five patients had two liver biopsies performed. Islets were identified in 80% of the patients (see Table 1). There was significant lipid deposition surrounding the islets in two cases and also significant immune infiltrate in these same two patients, which included B cells. Both patients were C-peptide positive with evidence of graft-function at time of biopsy. Three of the five patients remained off insulin including one where doses of their immunosuppression therapy were increased.

Conclusion: Islet transplantation remains a potential alternative to whole pancreas transplant in T1D patients. Three of the five patients remained off insulin including one where immunosuppression therapy was changed in response to biopsy findings. This project has identified physiological changes that occur within the liver after the islet transplantation. The biopsy findings led to changes in immunosuppressive therapy in 2 of 5 patients. Protocol liver biopsies are useful for research and may also guide therapy in some patients.

Table 1

		Islet found	Immune infiltrate	Lipid deposition	Immuno-suppression change
Patient 1	Biopsy 1	No	No	Yes	No
	Biopsy 2	Yes	Yes	Yes	Yes
Patient 2	Biopsy 1	No	No	No	No
	Biopsy 2	No	No	No	No
Patient 3	Biopsy 1	No	No	No	No
	Biopsy 2	Yes	Yes	Yes	No
Patient 4	Biopsy 1	Yes	No	Yes	No
	Biopsy 2	Yes	Yes	Yes	Yes
Patient 5	Biopsy 1	No	No	No	No
	Biopsy 2	Yes	No	No	No

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Cellular senescence in type 1 diabetic patients: effect of organ transplantation

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Background: Organ transplantation has become a common procedure in individuals with end-stage organ failure. Restoration of the organ function has been associated with improved life expectancy in many though not all organ transplantations. We have hypothesized that measurement of the length of telomeres, nucleoprotein complexes that serve as protective caps of linear eukaryotic chromosomes, could provide a marker for changes in cell senescence after transplantation procedures. Therefore, we have determined

telomere length (number telomeric repetitions/albumin copies number) and telomerase activity (by telomerase subunits assessment) in diabetic patients before and after pancreas transplantation.

Materials and methods: We have recruited 19 non-diabetics (ND) (43±16yrs old; 10M/9F; BMI 22.3±3.4 Kg/m²), 19 type 1 diabetes (T1DM: 47±11 yrs; 8M/11F; BMI 25.5±2.2 Kg/m², Diabetes duration 26.6±10.3 yrs, HbA1c 8.4±0.3%) and 27 T1DM from the waiting list for pancreas alone or pancreas after kidney transplantation (L-TX: 44±9 yrs; 13M/14F; BMI 23.9±3.2; Diabetes duration 26.4±9.7 yrs, HbA1c 8.2±1.6%) and 15 transplanted T1DM (TX: 42±6 yrs; 10M/5F; BMI 21.7±3.0; Diabetes duration 27.5±9.4 yrs, HbA1c 7.3±1.0%). In all patients anthropometric data were collected and blood was drawn for lab tests. Genomic DNA and total RNA were extracted from circulating nucleated blood cells. Telomere length was determined by Real-Time qPCR, while Real-Time RT-PCR was used for assessment of telomerase subunits HTERT (catalytic component) and HTERC (RNA component), and CCR2 gene expression.

Results: Telomere length (median value 0.126) was determined in all subjects. In the study population as a whole, telomere length was inversely associated with age ($r=0.240$, $p=0.035$), BMI ($r=0.258$, $p=0.041$), total cholesterol ($r=0.290$, $p=0.021$), LDL ($r=0.281$, $p=0.025$), and C-peptide ($r=0.315$, $p=0.007$) (all $p<0.05$ or less), while the correlation was positive with HDL ($r=0.610$, $p<0.001$), and duration of diabetes (DD) ($r=0.421$, $p=0.004$) (both $p<0.05$ or less). By regression stepwise followed by multiple regression analysis, DD and HDL remained independently associated with telomere length. When the different subgroups were examined, telomere length was higher in L-TX (0.217 ± 0.028) than in ND (0.153 ± 0.015 ; $p<0.05$). Moreover, HTERT gene expression was higher (0.0354 ± 0.0009 and 0.164 ± 0.029 , respectively) in T1DM and L-TX than in ND (0.010 ± 0.002 ; all $p<0.05$ or less). Upon normalization of telomere length by telomerase activity (T/T), a significant telomere shortening became apparent in T1DM and L-TX (8.07 ± 1.48 and 12.57 ± 0.49 , respectively) as compared to ND (248.9 ± 44.6 ; both $p<0.05$). TX patients had telomere length and T/T similar to that found in ND. Finally, CCR2 (MCP-1) receptor mRNA expression was greater in L-TX ($+549\pm100\%$; $p<0.001$) as compared to ND, while there was no difference in TX. CCR2 mRNA expression correlated with T/T values ($r=0.458$, $p=0.047$) and HTERT component ($r=0.896$, $p<0.001$).

Conclusions: Our data demonstrate an apparent effect of T1DM on markers of cellular senescence with telomere length being affected by duration of diabetes and HDL-cholesterol levels. Pancreas transplantation may reduce the process of telomere shortening. Assessment of telomere length may help risk stratification in T1DM and be a marker of outcomes of pancreas transplantation.

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Effect of tungstate on the survival and function of Langerhans islets transplanted into the anterior chamber of the mouse eye

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Background and aims: Islet transplantation is an alternative therapy to conventional ones for restoration of beta-cell function in type 1 and 2 diabetic patients. However, challenges remain related to the establishment of an appropriate immunosuppressive therapy and the ability to *in vivo* monitoring the transplanted islets. The anterior chamber of the eye was recently proposed as a privileged transplantation site for pancreatic islets, enabling non-invasive *in vivo* imaging, fast islet engraftment due to the high vascularization of the iris, and reduced graft rejection. Previous studies by our group have demonstrated that the treatment of diabetic animals with sodium tungstate salt (phosphatase inhibitor) is able to rescue the diabetic phenotype by increasing beta cell replication and diminishing apoptosis. Based on such observations, we hypothesize that the administration of tungstate to diabetic-induced mice transplanted with pancreatic islets into the anterior chamber of the eye should stimulate graft survival and function.

Materials and methods: After 3 days of culture following isolation from mice pancreas, 100-150 islets were transplanted into the anterior chamber of the eye of diabetic-induced mice (streptozotocin (STZ)-treated 8 days before transplantation). After transplantation, animals were divided into 4 group ($n=5$): WT - transplanted (T), treated with sodium tungstate (W) (0.5 mg/ml water); T - transplanted, untreated with tungstate; W- non-transplanted, tungstate-treated; C - control, non-transplanted, untreated with tungstate.

During 22 days of treatment, *in vivo* studies were performed to analyze glycemic levels, islet revascularization (staining with Evan's blue) and cell viability (staining with CFDA-SE cell tracer and propidium iodide). Post-mortem morphometric analysis and functional studies were conducted on graft-containing eyes and pancreas.

Results: Our results showed, for both WT and T groups analyzed, the efficient vascularization of the engrafted islets within 11 days and maintenance of islet viability and vasculature within 22 days after transplantation. Preliminary results showed decreased glycemic levels within 8 days after transplantation for the WT group when compared to the values obtained for the T and C groups (96 ± 14 vs 184 ± 38 and 351 ± 38 mg/dl, respectively). This tendency was maintained until the end of our study (96 ± 3 vs 245 ± 24 and 356 ± 42 mg/dl, for T and C groups respectively), suggesting the protective role of tungstate on the transplanted islets. These results are confirmed by observations of increased beta cell replication (expression of insulin and Ki67) and vascularization, and reduced cell death in the WT group when compared with the other groups analyzed, supporting the role of tungstate on graft survival.

Conclusion: Our results suggest that the combination of islet transplantation and tungstate treatment is the best strategy to achieve the reversion of the diabetic phenotype in STZ-diabetic mice by improving graft survival and recovery of endocrine function. Future work will be performed with human islets isolated from cadaver donors.

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Effect of tacrolimus versus cyclosporine on glucose metabolism of pancreas and kidney recipients in the late post-transplant period

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Background and aims: The aim of our study was to compare the glucose metabolism in type 1 diabetic recipients of kidney and pancreatic grafts on tacrolimus versus cyclosporine-based immunosuppression in conjunction with mycophenolate mofetil in the late posttransplant period.

Methods: We examined 26 insulin-independent patients after simultaneous pancreas and kidney transplantation with systemic venous drainage of pancreatic graft. All recipients had a stable good function of the kidney graft. Fasting glycemia, insulin levels, glycosylated hemoglobin (HbA_{1c}), a standard intravenous glucose tolerance test (IVGTT) with coefficient of glucose assimilation (K_{it}) calculation and trough Tacro/Cyclo levels were assessed. Insulin sensitivity was evaluated using the homeostasis model assessment (HOMA-IR). Total C-peptide and insulin secretions were calculated as areas under the curves from the serum levels during the IVGTT.

Results: Tacro and Cyclo groups did not differ in age, BMI and posttransplant period (9.7 ± 1.9 [SD] vs. 10.9 ± 1.3 years). We did not find any significant difference in response of IVGTT. In Tacro group (n=13) 3 patients had an abnormal response to glucose stimulus, 3 patients had an impaired glucose tolerance and 7 patients had a normal glucose tolerance. In Cyclo group (n=13) the abnormal response was present in none, the impaired glucose tolerance in 5 and the normal glucose tolerance in 8 recipients. The other results are shown in the following table.

	HbA _{1c} DCCT (%)	Fasting glycemia (mmol/L)	HOMA- IR	K _{it} (%/min.)	Total C-peptide secretion (pmol/ mL/60min.)	Total insulin secretion (mIU/L/60min.)
Tacro group	5.8 ± 0.41	5.05 ± 0.49	2.72 ± 2.64	1.24 ± 0.48	92 ± 47	1742 ± 1110
Cyclo group	5.7 ± 0.24	4.9 ± 0.55	2.26 ± 1.69	1.48 ± 0.53	98 ± 51	1440 ± 1093
Difference	NS	NS	NS	NS	NS	NS

Trough levels of calcineurin inhibitors had no significant impact on any of examined parameters.

Conclusion: The use of different types of calcineurin inhibitors in type 1 diabetic pancreas and kidney recipients had no effect on glucose metabolism in the late posttransplant period.

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Effects of dipeptidyl peptidase 4 inhibition with MK-0431 on syngeneic mouse islet transplantation

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Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibitors increase circulating levels of active incretin hormones-glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) by blocking their degradation. In addition to their insulintropic actions, GLP-1 and GIP also promote beta-cell proliferation and survival. The aim of this study was to test if DPP-4 inhibition with MK-0431 is beneficial for diabetic recipients with a marginal number of fresh islets.

Materials and methods: We syngeneically transplanted 150 C57BL/6 mouse islets under the kidney capsule of each streptozotocin-diabetic mouse, and then treated recipients with (n=20) and without (n=14) MK-0431 (30 mg/kg, po) for 6 weeks. Before and after transplantation, recipients' blood glucose, body weight and intraperitoneal glucose tolerance test (IPGTT) were measured. At 6 weeks, islet grafts were removed to determine insulin content and beta-cell mass.

Results: After islet transplantation, blood glucose levels decreased progressively in both groups. The mean (\pm SE) area under the curve of IPGTT at 2 weeks (43659 ± 2097 vs. 42318 ± 2679 mg/dl), 4 weeks (32088 ± 3450 vs. 31722 ± 3653 mg/dl) and 6 weeks (32201 ± 3564 vs. 26169 ± 3711 mg/dl) were not significantly different between MK-0431-treated mice and controls ($p > 0.27$). The mean (\pm SE) blood glucose at 6 weeks was 233 ± 36 and 263 ± 48 mg/dl in the MK-0431-treated group and controls, respectively ($p = 0.62$). MK-0431-treated group exhibited increased body weight over time (19.5 ± 0.5 to 21.4 ± 0.6 g, $p = 0.003$) and controls maintained their weight (18.5 ± 0.9 to 19.6 ± 1.0 g, $p = 0.58$), but the difference between two groups was not significant throughout the study period. At 6 weeks after transplantation, the mean (\pm SE) insulin content (4.24 ± 0.85 vs. 6.27 ± 0.33 IU, $p = 0.18$) and beta-cell mass (7.7 ± 1.6 vs. 4.9 ± 1.4 mg, $p = 0.20$) of grafts were comparable in the MK-0431-treated group and controls.

Conclusion: These results indicate posttransplant DPP-4 inhibition with MK-0431 in the diabetic recipient with a marginal number of fresh islets did not improve transplantation outcome.

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Liraglutide increases VEGF secretion in rat pancreatic islets

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Background and aims: The formation of microvascularization by capillary sprouting at the site of islet transplantation is crucial for survival of the graft. Vascular Endothelial Growth Factor (VEGF), a major angiogenic factor, may be a key protein in modulating the angiogenesis of islets after transplantation. Development of a pharmacological approach enhancing VEGF synthesis could improve islet graft survival. Liraglutide has been shown to decrease islets apoptosis and increase survival in cultured or transplanted islets. The mechanisms of its role in islets viability in culture and during transplantation have to be identified. The aim of this work was to study *in vitro* the effects of liraglutide on islet viability and its relation to angiogenesis via VEGF secretion.

Materials and methods: Previous studies have determined a protocol allowing the systematic evaluation of pharmacological molecules. Following this protocol, cultures of rats islets were incubated in presence of 1 and 10 μ M of liraglutide (50 fold higher than pharmacological concentrations used) during 12, 24 and 48h. The islet viability was evaluated using fluorescein diacetate/propidium iodure dying and functionality was determined by glucose test stimulation. Islets insulin-secretion was expressed as an index of stimulation (IS). VEGF secretion was determined by ELISA assay.

Results: Islets viability was 100% in controls and with liraglutide. Ten μ M of liraglutide induced a significant stimulation of VEGF secretion as early as 24h with 10.57 ± 3.55 vs control with 4.42 ± 1.09 pg of VEGF/ μ g of protein ($p <$

0.05, $n=4$), and was maintained after 48h. It only appeared after 48h with 1 μM liraglutide. Levels of secretion were respectively: 39.28 ± 14.81 with 1 μM , 53.60 ± 25.03 with 10 μM ; 13.63 ± 4.48 pg of VEGF/ μg of protein with controls, $p < 0.05$, $n=4$). At the same time, a significant stimulation of the insulin-secretion was observed at 24h of culture with 1 and 10 μM of liraglutide and controls with respectively 7.86 ± 1.78 and 8.17 ± 2.43 vs 4.27 ± 1.46 μg of insulin/g of protein ($n=4$, $p < 0.05$). The effect was maintained after 48h with 1 μM : 2.63 ± 1.64 and 10 μM : 6.28 ± 4.74 for liraglutide vs controls: 2.29 ± 1.05 μg insulin/g of protein, $n=4$).

Conclusion: *In vitro*, suprapharmacological concentrations of liraglutide had no toxicity and significantly stimulated VEGF secretion in islets. Also, insulin-secretion increased during the first 24h of culture. VEGF secretion could be one of the mechanisms involved in the improvement of islet viability during transplantation. Increased angiogenesis remains to be assessed.

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Vitamin D supports successful low-dose combination therapy with anti-CD3, cyclosporine A and islet transplantation in diabetic NOD mice

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Background and aims: Anti-CD3 treatment has been considered the holy grail of immune therapies to revert recent-onset type 1 diabetes (T1D), but recent clinical trials have revealed a major drawback of anti-CD3 mAb therapy, namely its narrow therapeutic window in which low doses are ineffective and high doses can be efficacious but cause side-effects. In light of these issues, strategies that sidestep these limitations while preserving or improving the therapeutic efficacy of anti-CD3 monotherapy are essential.

Materials and methods: We investigated whether combination therapy with a potent vitamin D analog and cyclosporin A enhances the therapeutic efficacy of anti-CD3 mAbs (145-2C11 whole IgG) in diabetes recurrence upon syngeneic islet transplantation in diabetic NOD mice

Results: This combination therapy was well-tolerated based on weight, bone and calcium parameters. Remarkably, combining all three agents at low, sub-therapeutic doses strongly restrained immune-mediated islet destruction and subsequent recurrence of diabetes (79.5 ± 18.6 days; $p < 0.01$), by far exceeding the therapeutic efficacy of each of the mono- (24.8 ± 7.3 days for anti-CD3) and duo- (25.5 ± 12.4 days for anti-CD3 + CsA) therapies, even though dual-therapies prevented primary graft non-function. Despite this clear improvement in clinical outcome, anti-CD3 treatment was accountable for most of the early alterations in T cell numbers, Treg frequencies and expression of inflammatory homing receptor in the triple combination therapy.

Conclusion: Based on these preliminary data, we nevertheless propose that VDR agonists should be considered as a dose-reducing agent in combination therapies for autoimmune diseases.

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A new method for human pancreatic cell purification

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Background and aims: Primary human beta cells have become indispensable in research on diabetes and obesity. In 2001, our team described FACS purification of viable human beta cells using the zinc-sensitive probe Newport Green. This technique has become routine worldwide in the last 5 years. However, shear forces inherent in this cell sorting technique can lead to low cell viability (<50%), poor RNA quality (28S/18S ratio <1.8), and low cell attachment in culture post sorting. Machine of the Year 2009, Laser-Enabled Analysis and Processing (LEAP[™], CynTellect Inc, USA) system was developed to address these limitations in cell purification, via laser-mediated in situ elimination of undesired cells in culture without physically manipulating the cells of interest. The aim of this study was to purify human insulin secreting pancreatic beta using the LEAP[™] (<http://www.cyntellect.com>).

Materials and methods: Briefly, human pancreatic islets (purity 61% \pm 10.3 : range 20–75%, $n=5$) were dissociated with Accutase[™] at 37°C into a single cell suspension, filtered and stained with the zinc chelator, Newport Green (Invitrogen) and Hoescht 33342. Cells were seeded on uncoated plates (384-

well C-lect[™] plates) or immobilized in Matrigel. Laser power and spot size were titrated to get optimal kill efficiency and minimal collateral damage. RNA integrity (% of full length GAPDH cDNA) was determined post sorting, and subsequent quantitative real-time RT-PCR.

Results: Starting cell preparations were 31.2% \pm 1.53 ($n=5$) Newport Green positive. After laser processing (3 iterations, 1000 shots/second) cells were 89.5% \pm 5.78 ($n=5$) Newport Green positive, corresponding to 100 cells/well. Laser processing did not impact RNA integrity (average length of GAPDH cDNA from laser processed cells as compared to control cells) nor cell viability.

Conclusion: The purification of Newport Green positive beta cells is feasible on LEAP[™] and high purity (90%) can be obtained, mRNA length is maintained in laser processed cells (i.e. no signs of RNA degradation), as is cell viability. The LEAP[™] provides the unique ability to purify small samples with far greater yield and purity than other technologies and is a new method allowing the study of pure human beta cells in culture.

PS 020 Inflammation and beta cell damage

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Cytokine-induced endoplasmic reticulum stress regulates nuclear factor-kappa-B activation in pancreatic beta cells

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Background and aims: Type 1 diabetes is characterised by the dysfunction and destruction of pancreatic β -cells which may be mediated by pro-inflammatory cytokines such as IL-1 β , TNF- α , and IFN- γ . Exposure of β -cells to these cytokines results in the activation of both endoplasmic reticulum (ER) stress and nuclear factor- κ B (NF κ B) signalling; but the significance of these responses in type 1 diabetes remains unclear. We investigated the role of ER stress in cytokine-induced NF- κ B signalling and β -cell destruction.

Materials and methods: β -cell responses to pro-inflammatory cytokines were examined in pancreatic β -cell lines and in mouse islets. Combination of IL-1 β (100U/ml), TNF- α (100U/ml), IFN- γ (250U/ml) was used for cytokine treatments. The chemical chaperone 4-phenylbutyric acid (PBA - 2.5 mM) was used to alleviate ER stress whilst pyrrolidinedithiocarbamate (PDTC - 50 μ M) was used to inhibit NF κ B activation. The role of the pro-apoptotic transcription factor CHOP in cytokine-induced β -cell toxicity was assessed by siRNA knockdown. β -cell death was measured using a cell death detection ELISA. NF κ B activity was examined in a β -cell line that stably expresses a luciferase reporter under the NF κ B promoter. Gene and protein expression changes were assessed by real-time PCR and Western blot.

Results: In MIN6 cells, exposure to cytokines resulted in increased cell death, elevated expression of ER stress markers, including CHOP, and activation of genes known to be regulated by NF κ B, including inducible nitric oxide synthase (iNOS), inhibitor of κ B- α (I κ B α), superoxide dismutase 2 (Sod2) and monocyte chemoattractant protein-1 (MCP-1/Ccl2). Unexpectedly, alleviation of ER stress with PBA caused a potentiation of cytokine-induced cell death, associated with further increases in cytokine-stimulated NF κ B-regulated gene expression. Cytokine-induced cell death or NF κ B-regulated gene expression were not affected by a knockdown of CHOP expression using siRNA, but they were completely inhibited when co-treated with the NF κ B inhibitor PDTC. Reporter assays demonstrated that PBA increased NF κ B activation, which was associated with a prolonged decrease in cytoplasmic I κ B α level. In addition, gene expression analysis showed that PBA treatment altered the downstream expression pattern of genes involved in the unfolded protein response (UPR), which is activated following ER stress to assist in its resolution. We also found in isolated mouse islets that alleviation of ER stress with PBA potentiated cytokine-induced cell death.

Conclusion: These data suggest a novel mechanism by which ER stress conferred by pro-inflammatory cytokines interacts with NF κ B signalling in β -cells, possibly by regulating the replenishment of its inhibitory protein I κ B α . The interface between UPR and NF κ B signalling pathways may be important in the regulation of β -cell survival in type 1 diabetes.

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TNF α reduces ATP-binding cassette transporter A1 gene expression via p38 MAPK γ , resulting the pancreatic beta cells dysfunction

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Background and aims: ATP-binding cassette transporter A1 (ABCA1) is a pivotal regulator of lipid efflux from cells to apolipoproteins and plays an important role in reverse cholesterol transfer. Recent report indicates that mice with specific inactivation of ABCA1 gene in β cells had markedly impaired glucose tolerance and defective insulin secretion. Pancreatic islets isolated from these mice demonstrated altered cholesterol homeostasis and impaired insulin secretion in vitro. These results establish a new role for ABCA1 gene in β cell cholesterol homeostasis and insulin secretion, indicating that cholesterol accumulation may contribute to β cell dysfunction in type 2 diabetes. On the other hand, TNF α is a proinflammatory cytokine produced by acti-

vated macrophages during the pathogenesis of diabetes. In the present study, we examined the effects of TNF α on ABCA1 expression in pancreatic β cells. **Materials and methods:** ABCA1 expression was examined by real-time polymerase chain reaction (PCR), western blot analysis, and reporter gene assay in both rat pancreatic islets and rat insulin-secreting cell line, INS-1 cells incubated with TNF α . We investigated the influence of the constitutively active form or dominant-negative mutant of p38 MAPK γ on ABCA1 expression by TNF α .

Results: TNF α suppressed ABCA1 protein expression dose-dependently in INS-1 cells. Real-time PCR analysis showed at significant reduction in the abundance of ABCA1 mRNA in response to TNF α in rat pancreatic islets. TNF α also decreased the activity of the luciferase reporter protein that was under the control of the ABCA1 promoter. Using several pharmacological inhibitors for the signal transduction pathways, the inhibitory effect of TNF α on ABCA1 promoter activity was abrogated by a specific inhibitor of p38 MAPK γ , but not for other isozymes. Activation of p38 MAPK γ by TNF α peaked after 15 min of exposure to TNF α . Furthermore, the constitutively active form of p38 MAPK γ decreased ABCA1 promoter activity in INS-1 cells. However, the dominant negative p38 MAPK γ abrogated the inhibitory effect of TNF α on ABCA1 promoter activity. When INS-1 cells were exposed to 10 nM TNF α , the insulin secretion or cholesterol ester content was decreased or increased, respectively, while the effects of TNF α on insulin secretion or cholesterol ester content were reduced in the cells treated with the ABCA1-specific siRNA.

Conclusion: Elevation of cholesterol levels in pancreatic islet cell reduces glucose-stimulated insulin secretion. This correlation is consistent with that between the reduction in insulin secretion and the elevation of pancreatic islet cell cholesterol levels in mice lacking β cell ABCA1, suggesting that cholesterol has a direct effect on reduction of β cell function. Our data showed that p38 MAPK γ mediates TNF α -suppressed transcription of the ABCA1 gene in pancreatic β cells, indicating that TNF α plays an important role in pancreatic β cells dysfunction. Activation of the p38 MAPK γ pathway by TNF α inhibits ABCA1 gene transcription, indicating that TNF α plays an important role in the lipotoxicity of pancreatic β cells.

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Siglec 7 improves beta cell function and survival by inhibition of cytokine expression and secretion

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Background and aims: Local inflammation, cytokine and chemokine production within pancreatic islets is detrimental for the β -cell survival and function. Siglecs (Sialic-acid binding immunoglobulin like lectins) are cell surface receptors expressed on haematopoietic cells which participate in immune responses. Their ligands, sialic acids, have prominent position, negative charge and widespread distribution and thus are involved in cell-cell interactions as well as pathogen and toxin binding. Our investigations revealed a cell-type specific expression of Siglecs in pancreatic islets, which is altered in diabetic conditions. Siglec 7, expressed in the β -cell, was downregulated in diabetes and its ligands were oppositely regulated.

Materials and methods: To investigate the role of Siglecs in β -cell function, Siglec 7 over-expression by plasmid transfection was carried out in isolated human islets followed by glucose stimulated insulin secretion (GSIS) and TUNEL assay. For investigating the cellular mechanisms, isolated human islets were treated with the diabetogenic conditions of elevated glucose (33.3 mM), palmitate (0.5 mM) and the cytokine mixture IL-1 β (2 ng/ml) and IFN- γ (1000 U/ml). Cytokine production and secretion was analyzed by RT-PCR and by the Meso Scale Discovery® multi array technology.

Results: Siglec 7 mRNA was markedly downregulated in islets isolated from patients with T2DM with poorly functional β -cells (70% downregulation vs non-diabetic control islets) as well as in pancreases from autopsy from patients with T2DM (85% downregulation vs non-diabetic control pancreases). Hence, we aimed to know if over-expression of Siglec 7 has a protective effect on β -cell function and survival. Indeed, plasmid over-expression of Siglec 7 increased GSIS 1.5-fold in human islets isolated from patients with T2DM. In non-diabetic islets, the GSIS was increased 2.5-fold under basal conditions. Also, glucose and palmitate induced 3.5 and 4% β -cell apoptosis respectively, which was prevented by Siglec 7 overexpression (50 and 40% reduction respectively compared to lacZ transfected control islets). To investigate the mechanisms of this protective effect of Siglec 7, the secretion and expression of IL-1 β , IL-6 and TNF α was analyzed in Siglec 7 overexpressing isolated hu-

man islets subjected to stimulation of cytokine expression by diabetogenic conditions. The combination of 33.3 mM glucose and 0.5 mM palmitate induced the secretion of cytokines IL-1 β (1.75-fold), IL-6 (4-fold) and TNF α (2-fold), compared to control islets ($p < 0.05$). Siglec 7 overexpression led to inhibition of IL-1 β and TNF α secretion to basal levels and to a 40% decrease in IL-6 secretion by these islets. Treatment with the cytokine mixture also increased accumulation of cytokines in the islet supernatants after 4 days: IL-1 β (19-fold), IL-6 (4.6-fold) and TNF α (1.7-fold), which was inhibited in Siglec 7 overexpressing islets. This was also confirmed on the mRNA level. In isolated human islets from patients with T2DM Siglec 7 overexpression caused a reduction in basal IL-1 β (40%) and TNF α (50%) expression, compared to LacZ treated islets ($p < 0.05$).

Conclusion: Our data suggest that Siglec 7 is expressed in pancreatic islets, downregulated in T2DM and improves β -cell function and survival by inhibiting deleterious cytokine production and secretion.

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Endosomal TLR3 activation by Coxsackievirus initiates strong immune response and death of beta cells

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Background and aims: Group B Coxsackievirus (CVB) infection of β -cells is associated with the development of diabetes. Intra-islet viral particles were detected in patients with T1DM and T2DM, but the mechanisms of a correlation between virus infection and diabetes progression are poorly understood. In this study we asked the question which intracellular signals are involved and which pattern recognition receptor (PRR) binds to CVB dsRNA.

Material and methods: Isolated human islets were infected with two different serotypes of CVB3 and 4. Replication of CVB was confirmed by immunostaining of viral protein 1 (VP1) and titration of islet lysate. CXCL10 secretion from the islets was measured by ELISA, CXCL10, IFN β , IFN γ , IL-1 β , TNF α , IL-6, MCP1, IL-8, RIG-I, TLR3, MDA-5 and PKR mRNA production by quantitative RT-PCR and β -cell apoptosis by double-staining for the TUNEL assay and insulin. Islet protein expression and phosphorylation were analyzed by western blot. Binding of CVB dsRNA to PRRs were investigated by the use of immunoprecipitation-RT-PCR coupled assays.

Results: Immuno- and TUNEL staining of infected islets revealed that β -cell apoptosis was 7-fold increased in CVB infected islets with a strong colocalisation of VP-1 and insulin positive β -cells. Immunoprecipitation-RT-PCR coupled assays showed that extracellular viral components bound to transmembrane-anchored, endosomal members of the Toll-like receptor family (TLR3) and transduced a signal via cytoplasmic adaptor proteins. The cytoplasmic receptor protein kinase R (PKR) mediated the induction of response genes from intracellular pathogen-produced substrates. In contrast, retinoic acid inducible gene I (RIG-I) and Melanoma differentiation associated protein 5 (MDA-5) were not involved in the recognition of CVB's dsRNA, despite the upregulation of RIG-I and MDA-5 mRNA in islets. TLR3 sensing resulted in increased mRNA levels of CXCL10, IFN β , IL-1 β , TNF α , IL-6, MCP1 and IL-8 in the infected islets. Among them, CXCL10 was the highest induced factor (~35-fold increase in secretion, 15-fold increase in mRNA, $p < 0.001$), compared to uninfected islets. In contrast, IFN γ remained unchanged; underlining that CXCL10 upregulation was TLR3 dependent. Additionally mRNA levels of the PRRs TLR3, RIG-I, MDA5 and PKR which are responsible to sense viral RNA were increased during infection resulting in amplified immune response. Analysis of survival and apoptotic pathways showed that the protein Akt was acutely activated during CVB infection and lasted for several hours, but switched to pro-apoptotic signaling at 24h p.i. with Akt downregulation, activation of pJNK and Caspase-3 and the appearance of the viral protein VP1.

Conclusion: Our data show that CBV infections have a direct deleterious effect on β -cell survival, resulting from virus-induced activation of pro-inflammatory cytokines and chemokines and upregulation of PRR mRNA triggered by TLR3 binding of CVB dsRNA. During the early phase of infection, the virus triggers the Akt pathway, possibly to initially maintain host survival and its own replication. Further understanding of the pathways involved in viral infection of β -cells will be of particular interest in order to develop new therapies to rescue the β -cell.

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Prevention of multiple low dose streptozotocin (MLD-STZ) induced diabetes in mice by extract from gum resin of boswellia serrata and 11-keto-B-boswellic acids

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Background and aims: Type 1 diabetes and LADA are autoimmune diseases where a chronic inflammatory process destroys insulin producing β -cells. Boswellic acids which derive from the gum resin of Boswellia species have been shown to possess immunomodulatory properties and suppress proinflammatory cytokines including NF κ B. In the model of MLD-STZ diabetes which is thought to correspond to human type 1 diabetes it was tested whether or not an extract of the gum resin of Boswellia serrata (BE), 11-keto- β -boswellic acid (KBA) and acetyl-O-11-keto- β -boswellic acid (AKBA) could prevent increase of proinflammatory cytokines in the blood, insulinitis and subsequent hyperglycemia.

Methods: BK+/+ male mice were i.p. injected with 40 mg/kg STZ for 5 days. A second group received 150 mg/kg BE, a third group 15 mg/kg AKBA and a fourth group 7.5 mg/kg KBA simultaneously over a period of 10 days. Infiltration of lymphocytes into pancreatic islets and apoptosis of islet cells were determined histochemically (CD-3 antibodies and anticaspase-3) after day 10. In addition, proinflammatory cytokines were determined in serum. Blood glucose was measured over a period of 35 days.

Cytokines	Controls	STZ	STZ+BE	STZ+AKBA	STZ+KBA
G-CSF	28.44 \pm 3.39	74.816 \pm 12.60***	28.829 \pm 3.47**	8.323 \pm 2.08***	19.3 \pm 3.6***
GM-CSF	0.79 \pm 0.036	2.97 \pm 0.22***	1.610 \pm 0.430**	1.02 \pm 0.01***	1.10 \pm 0.29***
IL-1A	1.89 \pm 0.27	5.18 \pm 0.37***	2.65 \pm 0.38***	1.93 \pm 0.11***	2.04 \pm 0.28***
IL-1B	3.13 \pm 0.6	10.01 \pm 1.06***	4.44 \pm 0.75**	2.33 \pm 0.09***	3.35 \pm 0.56**
IL-2	1.31 \pm 0.19	7.55 \pm 0.84***	2.92 \pm 0.79**	1.16 \pm 0.04***	2.38 \pm 0.51**
IL-6	7.61 \pm 1.55	17.55 \pm 2.61***	8.62 \pm 1.53*	9.28 \pm 0.28**	6.41 \pm 1.22**
TNF- α	1.37 \pm 0.23	3.45 \pm 0.25***	1.31 \pm 0.22***	1.09 \pm 0.08***	1.88 \pm 0.45*
IFN- γ	2.98 \pm 0.96	8.61 \pm 1.55*	2.92 \pm 0.9**	1.95 \pm 0.05**	1.89 \pm 0.33**

Results: 10 days after first STZ injection there was infiltration of lymphocytes into pancreatic islets and appearance of apoptotic cell. Moreover, all tested proinflammatory cytokines in the serum were significantly increased. There was also a continuous increase of blood glucose. Simultaneous i.p. administration of BE, AKBA and KBA significantly reduced cytokines in the serum, infiltration of lymphocytes and apoptosis. After 21 days blood glucose levels showed no increase.

Conclusion: In MLD-STZ-treated mice an extract of the gum resin of Boswellia serrata and two of its constituents i.e. AKBA and KBA prevented development of diabetes probably through their inhibitory action on proinflammatory cytokines.

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Reg1 and Reg3 β expression in the pancreas of adult diabetic Goto-Kakizaki rats

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Background and aims: Reg protein expression is associated with islet development, β -cell damage, diabetes and pancreatitis. We previously reported that islets of 4-month-old (4-mo) Goto-Kakizaki (GK) rats, a spontaneous model of type 2 diabetes, overexpress Reg1, 3 α , 3 β and 3 γ vs age-matched Wistar control islets. Reg1 and 3 β are involved in cell growth/survival control and inflammation, respectively. Diabetic GK islets also exhibit progressive inflammatory reaction, consisting of high CCL2 (MCP-1), CCL3 (MIP-1 α), CXCL-1 (murine IL-8 analog) and IL-6 expression/release and mostly peri-islet mac-

rophage infiltration. Importantly, Reg1 gene promoters contain IL-6-responsive elements. Here we analyzed in greater detail the pancreatic expression of Reg1 and Reg3 β in diabetic GK rats.

Materials and methods: Isolated pancreatic islets and acinar (exocrine) tissue from male Wistar and GK rats were used for quantitative RT-PCR analysis, normalized with GAPDH. Islet IL-6, CCL2, CCL3 and CXCL1 release was measured by Luminex™ technology after a 48h islet culture on collagen. Pancreatic Reg immunohistochemistry (IHC) was performed on paraffin sections with a rabbit anti-human polyclonal antibody (Ab) and a mouse anti-rat monoclonal anti-Reg1 Ab. Macrophage infiltration was detected on cryostat sections using CD68 and MHC class II antibodies. Islet macrophage⁺ area was quantified and expressed as % of corresponding islet area. Statistical analyses used the Student's t-test for unpaired data.

Results: The exocrine/endocrine ratio of Reg mRNA expression in 4-mo normoglycemic Wistar and diabetic GK rats was: 1) Reg1: 41.3 \pm 2.4 and 5.0 \pm 1.5, respectively (n=3 different isolations/group, p<0.005); 2) Reg3 β : 74.2 \pm 17.0 and 7.0 \pm 4.9, respectively (n=3, p<0.02). Furthermore, Reg1 and 3 β were overexpressed in GK vs Wistar islets (x11.2 \pm 1.4, p<0.005 and 77.9 \pm 16.3, p<0.01, respectively, n=3 in both cases). Next, the polyclonal Reg Ab stained most islet insulin⁺ cells in 4-mo Wistar pancreas but much less so in GK pancreas. By contrast, the monoclonal anti-Reg1 Ab stained just a few peri-islet exocrine cells in Wistar pancreas but many more in GK rats, particularly around large islets. Since IL-6 stimulates Reg1 expression, we compared cytokine/chemokine release by small and large 2.5-mo Wistar and GK islets. Large GK islets released significantly more IL-6 and CCL3 than large Wistar islets (x4.7 \pm 0.9, p<0.02 and 2.7 \pm 0.2, p<0.005, respectively, n=3 in both cases). Concomitantly, CD68⁺ and MHC II⁺ peri-islet macrophage infiltration correlated with islet size in 2.5-mo GK rats (r=0.57 and r=0.95, respectively).

Conclusion: Reg1 and 3 β genes are strongly expressed in the exocrine pancreas of control rats. They are markedly overexpressed in islets of diabetic GK vs control Wistar rats, probably reflecting an adaptive stress response. While the polyclonal Ab mainly stains islet insulin⁺ cells, Reg1 protein is the hallmark of acinar tissue around large GK islets. Its peri-insular localization might result from increased islet release of IL-6 (a factor stimulating Reg1 expression) and is similar to that of macrophage infiltration.

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A network based approach for identification of proteins involved in beta cell failure in type 1 diabetes

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Background: Type 1 diabetes (T1D) is characterized by absolute insulin deficiency due to immune-mediated destruction of the insulin-producing pancreatic β -cells. Recent T1D genome-wide association studies have established a large number of loci contributing to T1D risk. Most causal genes within these loci are, however, still unknown and need to be identified.

Aim: To predict novel candidate genes/proteins important in T1D pathogenesis by the use of a bioinformatics approach and subsequent experimental *in vitro* validation.

Methods and Results: All positional candidate genes from 44 non-HLA T1D-associated regions were used to generate protein-protein interaction networks using the STRING software. The identified networks were further prioritised by detailed SNP enrichment analysis and expressional profiling in human pancreatic islets exposed to pro-inflammatory cytokines (IL-1 β , IFN γ and TNF α). Three networks consisting of 117 nodes in total were significantly enriched for associated SNPs and transcriptional regulation in human islets. Seventy-two of the 117 proteins could be categorized as either cell membrane- or secreted proteins. Of these, seventeen genes were significantly (p<0.05, n=8) upregulated by more than 1.5-fold or downregulated by at least 0.85-fold by cytokine treatment compared to baseline. Further expressional profiling substantiated that 14 of these genes are also expressed and differentially regulated upon cytokine stimulation in both primary rat islets and in the rat beta-cell line INS-1. These genes included the chemokines CXCL9, CXCL10 and CXCL11 and several cytokines such as IL15 and IL18, and the membrane bound proteins CXCR7, CD83 and CD276.

Conclusion: We have by an unbiased integrative network approach identified 14 proteins appearing in disease networks of type 1 diabetes which potentially play important roles in the failure of beta-cells. Most of the identified genes are known as immune genes, however, we show here that these genes are expressed and differentially regulated in a beta-cell line. Functional char-

acterization studies of these proteins in beta-cells and islets will establish the potential impacts on beta-cell failure.

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PTBP1 and translation of diabetogenic viruses in beta cells

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Background and aims: Glucose entry in the pancreatic beta cell triggers insulin secretion and the rapid biosynthesis of insulin granules (ISGs). We have shown that glucose and cAMP independently promote the nucleocytoplasmic translocation of polypyrimidine tract-binding protein-1 (PTBP1) in beta cells. Cytosolic PTBP1 binds the mRNAs encoding ISG proteins, thus enhancing their stability and translation. PTBP1 can also foster the IRES-mediated translation of picornaviruses, including enteroviruses. Several prospective studies have suggested that enterovirus infection may trigger type 1 diabetes. Thus, we investigated whether diabetogenic enteroviruses hijack the machinery for ISG biogenesis, and in particular PTBP1, for their effective propagation in beta cells.

Materials and methods: We used two different strains of Echovirus 9 (EV-9) to investigate whether PTBP1 is involved in the viral translation, the diabetogenic EV-9 DM and EV-9 Barty isolated from a nondiabetogenic child. We performed *in vitro* RNA binding assay and dual luciferase reporter assay as well as direct infection of INS-1 cells after knockdown of PTBP1.

Results: We could show that PTBP1 binds to the 5'-UTR of the diabetogenic EV-9 DM. The 5'-UTR of EV-9 DM is more efficient in promoting translation than the 5'-UTR of the non-diabetogenic EV-9 Barty. We found that glucose stimulation of insulinoma cells increases the translation of EV-9 DM in a PTBP1-dependent fashion.

Conclusion: Taken together, our findings support the hypothesis that diabetogenic enteroviruses exploit PTBP1 for their translation in beta cells, and thus may affect the biosynthesis of ISG components.

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PS 021 Fat islets: lipotoxicity *in vitro* and *in vivo* models

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Endoplasmic reticulum stress precedes diabetes and its associated changes in differentiation and inflammation in db/db mouse islets

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Background and aims: Type 2 diabetes (T2D) is accompanied by the dysfunction and destruction of pancreatic β -cells. This is associated with a loss of the unique expression pattern of genes that maintain β -cell differentiation and optimise glucose-stimulated insulin secretion (GSIS). Endoplasmic reticulum (ER) stress and inflammation have been proposed as potential mechanisms. However the temporal changes and the significance of these responses in the genesis or progression of T2D remain unknown. Here, we investigated the time-dependent gene expression changes in islets of db/db mice during their progression to diabetes.

Materials and methods: Pre-diabetic (5–6 weeks of age) and obese diabetic (12 weeks of age and above) db/db mice and age-matched db/+ (control) mice were studied. Glucose tolerance was assessed using i. p. glucose tolerance test. Islets were isolated and RNA extracted for gene expression analysis by real-time PCR.

Results: Pre-diabetic db/db mice displayed increased body weight and glucose intolerance compared to control mice. The most striking changes in gene expression in pre-diabetic db/db mouse islets were the highly elevated mRNA levels of multiple ER stress-inducible unfolded protein response (UPR) genes (spliced Xbp1, Atf4, Bip, Edem1, Erp72, Fkbp11, Grp94, p58, and Herpud1). Interestingly, the expression of most of these genes declined in diabetic db/db mice (with the exceptions being Edem1 and Herpud1 that are involved in ER-associated degradation). This suggests that the UPR may be maximally activated prior to the onset of diabetes in db/db mice. In contrast, expression of metabolic and secretagogue receptor genes were either unchanged (mGPR40, GPR40 and Gpr1r) or modestly reduced (Glut2, PC and Gpr) in pre-diabetic mice, whereas they were markedly reduced in diabetic mice. Similar patterns of time-dependent reductions in gene expression were observed for several transcription factors important for the maintenance of β -cell differentiation (Beta2, MafA, Nkx6.1 and Pdx1). On the other hand, anti-oxidant (Catalase, GPx and HO-1) and inflammation (Fas, Gro-1, IL-1 β , IL-6, Lipocalin2 and TNF α) genes displayed time-dependent increases in expression. Interestingly, the expression of a gene capable of inhibiting differentiation, Id1 was significantly increased prior to diabetes onset, and remained elevated in diabetic mice.

Conclusion: These data indicate that maximal changes in ER stress gene expression precede diabetes in db/db mice. In contrast, the major reductions in the β -cell differentiation genes accompany diabetes, as do the increases in inflammatory and oxidative stress gene expression. These findings suggest that ER stress is an early event in the maladaptation of β -cells during the progression to T2D and that the UPR may play a role in the loss of β -cell differentiation.

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The islet inflammation and fibrosis precede structural and functional abnormalities of the pancreatic islets in obese diabetic db/db mice

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Background and aims: The BKS.Cg- $Lepr^{db}/+$ $Lepr^{db}/Jcl$ (db/db) mice develop severe hyperglycemia with marked insulin resistance and limited capacity of insulin secretion. On the other hand, the B6.V- $Lepr^{ob}/J$ (ob/ob) mice with a similar genetic background do not present severe hyperglycemia because of a compensatory hypersecretion of insulin. Previously, we demonstrated that the islet fibrosis is more significant in db/db mice than in ob/ob mice with the comparable islet function at an early stage of life. In this study, to further clarify the molecular mechanism that induces the pancreatic islet dysfunction in db/db mice, we compared a comprehensive gene expression profiles, function and structure in db/db with those in ob/ob mice.

Materials and methods: The pancreatic tissue sections and isolated islets from male db/db and ob/ob mice at the age of 6 weeks, when pancreatic islet function and structure were comparable, were applied to gene expression analysis by using real time RT-PCR with Sybr Green. Gene expression was quantified by the comparative Ct method with each result corrected by 18S rRNA quantity. The immunohistochemical analyses by using anti-F4/80, cluster of differentiation (CD)4 and CD8a monoclonal antibody were studied to reveal the degree of infiltration of inflammatory cell into the islets. To confirm the remaining of leptin signal in db/db mouse islets, we analyzed inflammatory cytokine-related gene expressions in the isolated islets by addition of recombinant mouse leptin (0, 25, 50, 100 ng/ml). The existence of functional short-form leptin receptor (Ob-Rs) in the islets was assessed by laser capture microdissection method and real time RT-PCR.

Results: The gene expressions associated with inflammatory cytokines and profibrosis were significantly upregulated in db/db compared with ob/ob mice (il1 β : 2.5×10^{-4} vs. 3.3×10^{-5} , tgfb β 1: 3.1×10^{-4} vs. 1.1×10^{-4} , collagen, type 1, α 1 (col1a1): 1.0×10^{-3} vs. 4.9×10^{-5} , collagen, type 3, α 1 (col3a1): 1.0×10^{-3} vs. 8.0×10^{-5}). In contrast, there was no difference in the mRNA level of matrix metalloproteinase 9 (mmp9), which is a type of collagenase, between two mouse strains. Consisted with the result of cd68 gene expression (db/db mice: 5.8×10^{-5} vs. ob/ob mice: 1.7×10^{-5} , $p < 0.05$), F4/80-positive ratio in the islet was higher in db/db than in ob/ob mice ($15.6 \pm 3.1\%$ vs. $7.4 \pm 0.9\%$, $p < 0.05$). The F4/80-positive cells were mainly observed in the mantle area of islets which prone to become fibrosis. Corresponding to the result of cd4 and cd8a gene expressions, both CD4 and CD8a cell positivity in the islet was comparable and very few in two strains of mice. The il1 β , il6 and tgfb β 1 gene expressions in isolated islets were upregulated dose-dependently in db/db mice, as well as in ob/ob mice, by addition of recombinant mouse leptin. The functional Ob-Rs was present in the islet core area mainly containing beta-cells.

Conclusion: The islet inflammation and fibrosis precedes the deterioration of pancreatic islet structure and function in db/db mice by the remaining of leptin signal through the functional Ob-Rs.

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FABP5 depletion protects from gluco- and lipotoxicity in beta cells

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Background and aims: Fatty acid binding proteins (FABPs) facilitate the uptake, transport and metabolism of fatty acids within the aquatic environment of the cell. FABP5 (mal1, E-FABP) was previously reported to be expressed in rat islets and in the β -cell line INS1E of rat origin. Subsequently, we asked the question if FABP5 is also expressed in human and mouse islets and whether it regulates β -cell survival and function.

Material and methods: β -cell survival and function was monitored using double-staining for TUNEL and insulin and by glucose stimulated insulin secretion assays on isolated islets from FABP5-/- and wildtype mice at high fat/ high glucose diet (HFD) for 16 weeks. In another set of experiments, islets were treated with high levels of glucose (22.2 mM), palmitate (0.5 mM) or a mixture of IL-1 β (1 μ l/ml) and INF γ (1000 U/ml) for three days. These conditions were also applied to the human β -cell line CM9 and the rat β -cell line INS1E. FABP5 was silenced using siRNA and survival was analyzed by Western Blotting.

Results: FABP5 was expressed in human and mouse islets. Oppositely to previous reports in rats, FABP5 expression was restricted to α -cells. Since FABP5 is known to mediate the inflammatory response in macrophages, we exposed isolated islets from FABP5-/- and wildtype mice to a pro-diabetic milieu. While 3-day exposure of wildtype mice to elevated glucose concentrations, palmitate and the cytokine mix increased β -cell apoptosis (3.6-, 7.5- and 10-fold, respectively) and impaired glucose stimulated insulin secretion (1.4-, 1.8- and 2.1-fold, respectively). FABP5-/- islets were not affected by the treatments. No differences between FABP5-/- and wildtype islets were observed in control islets. After feeding FABP5-/- and wildtype mice a HFD for 16 weeks, islets were isolated and β -cell survival was analyzed. FABP5 -/- mice showed a 47% reduction in apoptotic β -cells compared to wildtype mice ($p < 0.05$). While FABP5 in mouse and human islets was only expressed in α -cells, the human CM9 and rat INS1E insulinoma β -cell lines expressed FABP5. 2-day treatment of both cell lines with elevated glucose, palmitate or the cytokine mixture induced cleavage of PARP and Caspase in control cells, in contrast silencing of FABP5 using siRNA prevented such effects, indicating improved survival in FABP5 depleted cells.

Conclusion: Our data show that depletion of FABP5 improved β -cell function and survival under diabetogenic conditions. This was mediated either di-

rectly in the β -cell in cell lines or by the crosstalk of α - and β -cells in isolated islets. Since we found FABP5 expressed in α -cells these results suggest a role of this lipid chaperon in α -/ β -cell interactions during diabetes progression.
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Palmitoleate levels and palmitate-induced lipotoxicity in beta cells

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Background and aims: Type 2 diabetes mellitus (T2DM) develops as a consequence of deteriorating β -cell function and insulin resistance. Chronically elevated levels of free fatty acids are known to affect the β -cells and thereby contribute to the pathogenesis of obesity-related T2DM. In vitro different effects of long-chain saturated and monounsaturated fatty acids on β -cell function have been described. Palmitate (C16:0) mediates lipotoxic effects including inducing β -cell apoptosis and impairing secretory function, whereas palmitoleate (C16:1) has been connected with cytoprotection. Indeed, when the two fatty acids were combined the negative effects of palmitate (PA) were counteracted by the addition of palmitoleate (PO). The aims of the present study were to determine PO/PA ratios in young individuals belonging to the Uppsala Longitudinal Study of Childhood Obesity (ULSCO) and in lean controls and use the values obtained for in vitro experiments. Finally we wanted to compare the effects of the fatty acids on β -cell function in vitro with the in vivo findings.

Material and methods: Fasting blood samples were obtained from 40 obese children, belonging to the ULSCO, and 13 lean controls. The children were 3 to 17 years old. All were normoglycemic. PO and PA levels were quantified by GS/MS. Insulin-producing cell lines MIN6 and INS-1E were treated with the same concentration of PA (0.25 mM) for 24 hours. To investigate the cytoprotective effect of PO, the monounsaturated fatty acid was added together with PA at three different ratios of PO/PA (1:2, 1:5, 1:10). Thus, concentrations of PO were 0.125, 0.05 and 0.025 mM, respectively. Glucose-stimulated insulin secretion (GSIS) was conducted after 24 hours. Secreted insulin was quantified by ELISA and normalized to DNA.

Results: In obese subjects belonging to ULSCO the ratio of PO/PA ranged from 1:2 to 1:10 with a mean ratio of 1:5. Interestingly, similar ratios between the fatty acids were determined in lean control subjects. When cells were cultured with the different PO/PA ratios the highest ratio between the fatty acids maintained a favorable secretory function in comparison to cells cultured at the same PA concentration but with lower levels of PO. After 24 hours treatment, cells cultured with the highest PO/PA ratio had low levels of basal insulin secretion and accentuated response to high glucose. In cells cultured with the lower ratios of PO/PA less difference between basal and stimulated insulin secretion was observed.

Conclusion: PO/PA ratios are not significantly different in obese subjects compared to lean. The cytoprotective effects of a high PO/PA ratio on β -cell function demonstrated in vitro, should be interpreted with caution with regard to implications for development of β -cell dysfunction in young obese subjects.

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Ceramide generation pathways in palmitate-induced beta cell dysfunction and apoptosis

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Background and aims: During development of type 2 diabetes mellitus high blood lipid levels in combination with hyperglycaemia lead to beta-cell dysfunction and apoptosis. One of the proposed mechanisms of how free fatty acids negatively affect the beta-cell is change in the metabolism of sphingolipids leading to increased generation of ceramide. The sphingolipid metabolites play important roles in the regulation of cell proliferation and survival but also cell death. Whereas ceramide inhibits proliferation and promotes apoptosis, other sphingolipids promote proliferation and are anti-apoptotic. Ceramide can be generated by de novo synthesis by adding palmitate to L-serine catalyzed by serine palmitoyltransferase (SPT). Additional pathways of ceramide generation include hydrolysis of membrane sphingomyelin by sphingomyelinases (SMases) and conversion of sphingosine by ceramide synthetases (CerS). In conditions with elevated fatty acid levels such as obesity

ceramide formation may significantly influence beta-cell fate. The aim of the study was to determine what pathway(s) of ceramide synthesis contribute to beta-cell dysfunction and apoptosis in cells exposed to palmitate.

Materials and methods: Insulin-secreting mouse insulinoma (MIN6) cells were cultured for 48 hours in the absence or presence of 0.25 mM palmitate. Ceramide generation was inhibited by ISP-1 (SPT), GW4869 (SMases) and Fumonisin B1 (CerS), which were included or not during culture. After culture, apoptosis and glucose-stimulated insulin secretion (GSIS) were determined by ELISA, and ceramide levels by thin layer chromatography.

Results: Prolonged exposure of MIN6 cells to palmitate resulted in rise in total ceramide levels and an almost 3-fold increase ($P < 0.05$) in apoptosis. Cells exposed to palmitate had lowered GSIS compared to control cells. Inhibition of ceramide de novo synthesis by ISP-1 had no effect on either apoptosis or GSIS in palmitate-treated cells. The level of ceramide was decreased, however. Suppression of sphingosine conversion to ceramide by Fumonisin B1 decreased palmitate-induced apoptosis by 33% and lowered ceramide levels. Despite these effects no improvement in GSIS, in comparison to palmitate-treated cells, was observed. Ceramide generation from sphingomyelin in palmitate-treated MIN6 cells was inhibited by GW4869. The level of apoptosis was decreased by 29% compared to palmitate alone and ceramide levels were decreased. Again, these positive effects did not improve the impaired GSIS observed in cells treated with palmitate. Finally, when Fumonisin B1 and GW4869 were delivered together to palmitate-treated cells, apoptosis level was decreased by 46% and almost normalised. Also, ceramide amounts were substantially lowered. Despite this effective way of counteracting ceramide build-up the combined administration was not able to improve the insulin secretory characteristics.

Conclusion: Palmitate is inducing ceramide production in beta-cells via primarily sphingomyelin cleavage and conversion of sphingosine. Whereas ceramide synthesis seems to play a major role in palmitate-induced beta-cell apoptosis, it has no effect on restoring the impairments in the insulin secretory machinery induced by the fatty acid.

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Transgenic mice with beta cell-specific p8 overexpression preserve insulin secretory function under high fat diet-induced lipotoxic beta cell stress

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Background and aims: The intracellular protein p8 is acutely induced by pancreatitis and protects exocrine acinar tissue from inflammatory damage. Within the endocrine pancreas, own previous work has demonstrated in vitro that p8 is a glucose-induced mediator of beta cell proliferation and reduces STZ-induced beta cell apoptosis in an acute and transient manner. Here we investigated transgenic mice with pancreatic beta cell-specific p8 overexpression under basal conditions and high fat diet (HFD)-induced lipotoxicity.

Materials and methods: C57Bl/6NcrJ transgenic (tg) mice with beta cell-specific p8 overexpression were generated by pro-nucleus injection of a rat insulin promoter I driven p8 expression cassette. Tg mice and wt littermates were fed with normal and HFD chow for 10 weeks. Then unfasted serum insulin levels were measured and insulin function was tested by intraperitoneal glucose tolerance test (ipGTT) and insulin tolerance test (ipITT). Weight and blood were measured weekly. After experiments pancreases were removed for analysis of beta cell mass.

Results: Basal characterisation - 14 week old tg mice display about 5 fold (median) enhanced p8 gene expression in whole pancreas but not in other organs tested so far (liver, kidney). This confirms beta cell-specific expression of ectopic p8. Tg mice have a tendency to gain more weight but this is only significant in normal diet fed animals at week 10 when weight of tg animals comes close to that of HFD fed wt mice (27 vs. 28.5 g). No differences were observed between non-fasted blood glucose levels of tg vs. wt mice. Functional characterisation - Wt and tg mice do not differ regarding glucose tolerance (ipGTT), insulin resistance (ipITT) and non-fasted serum insulin. Upon HFD both wt and tg animals develop insulin resistance with substantially elevated non-fasted insulin levels. Tg mice demonstrate significantly enhanced glucose tolerance which is associated with enhanced beta cell mass. We expect that differences in beta cell mass, actually $p = 0.5688$ ($n = 5$) will become significant after analysis of the remaining mice.

Conclusion: Endogenous p8 is a beta cell proliferative and protective protein that is acutely upregulated upon beta cell injury in vivo. Transgenic beta cell specific overexpression of p8 improves insulin secretory function in response

to HFD-induced lipotoxic beta cell stress. p8 may represent a switch protein linking glucose-induced proliferation with anti-apoptotic signalling upon cellular stress.

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Excessive food intake, obesity and inflammation process in Zucker fa/fa rat pancreatic islets

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Background and aims: Inappropriate food intake-related obesity and more importantly, visceral adiposity, are major risk factors for the onset of type 2 diabetes. Evidence is emerging that nutrient-induced β -cell dysfunction could be related to indirect induction of a state of low grade inflammation. Our aim was to study whether hyperphagia associated obesity could promote an inflammatory response in pancreatic islets leading to β -cell dysfunction.

Materials and methods: In the hyperphagic obese insulin resistant male Zucker rat, we measured the level of circulating pro-inflammatory cytokines by Chemiarray rat cytokine kit from Chemicon, and Bio-Plex assay (Bio-Rad, Hercules) and estimated their production as well as the expression of their receptors in pancreatic β -cells using qPCR and immunofluorescence staining. Then, to get insight into the mechanisms involved in phenotypic alterations, Panorama antibody microarray (abArrays) (Sigma-Aldrich) were used to determine the expression profile of proteins implicated in different membrane receptors signaling, apoptosis, cell cycle, cytoskeleton, nuclear signaling, neurobiology. Finally, we have studied the effect of an exposure to IL-1 β of fa/fa and fa/+ rat islets on insulin release and β cells apoptosis.

Results: Our main findings concern intra-islet pro-inflammatory cytokines from fa/fa rats: IL-1 β , IL-6 and TNF α expressions were increased; IL-1R1 was also over-expressed with a cellular redistribution also observed for IL-6R. Ab Array analysis had showed that despite JNK overexpression, cell viability was unaffected probably because of decreases in cleaved caspase3 as well as in SMAC/DIABLO and APP, involved in the induction and amplification of apoptosis. Concerning β -cell proliferation, decreases in important cell cycle regulators (Cyclin D1, p35) and increased expression of SMAD4 probably contribute to counteract and restrain hyperplasia in fa/fa rat islets. Finally and probably as a result of IL-1 β and IL-1R1 increased expressions with sub-cellular redistribution of the receptor, islets from fa/fa rats were found more sensitive to both stimulating and inhibitory concentrations of the cytokine; this confers some physiopathological relevance to a possible autocrine regulation of β -cell function by IL-1 β .

Conclusion: These results support the hypothesis that pancreatic islets from prediabetic fa/fa rats undergo an inflammatory process. That the latter could contribute to β -cell hyperactivity/ proliferation and possibly lead to progressive β -cell failure in these animals, deserves further investigations.

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Epigenetic and functional alterations induced by palmitate in the insulin-secreting clonal beta cell line INS-1 832/13

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Background and aims: Type 2 diabetes is caused by a combination of insufficient insulin release and insulin resistance in target tissues. Adiposity and high levels of circulating free fatty acids (FFAs) are associated with Type 2 Diabetes and beta-cell dysfunction such as impaired insulin secretion. The cellular mechanisms behind the perturbations of insulin secretion induced by lipotoxicity are of yet not fully understood. Previous studies have revealed an intricate relationship between epigenetic modifications and gene expression in human islets. We aim to examine the link between epigenetic marks, gene expression and beta-cell function and in lipotoxic beta-cells.

Materials and methods: The clonal beta-cell line INS-1 832/13, exposed to 0.5 mM Palmitate for 48h, was used to evaluate the effect of lipotoxicity on insulin secretion, oxygen consumption rate (OCR), gene expression and histone modifications. To assess gene expression and histone modifications, TaqMan real-time PCR with gene-specific probes, histone acetyltransferases (HAT) and histone deacetylases (HDAC) activity assays and ChIP for 3 histone marks (activating marks H3Ac and H3K4me3 and closing mark H3K27me3) were used.

Results: Exposure to 0.5 mM palmitate significantly decreased insulin secretion evoked by 16.7 mM glucose in INS-1 832/13 beta-cells ($p=0.046$). While 16.7 mM of glucose increased the OCR 1.36 fold in control cells, an 1.08-fold increase in OCR was generated after 1h in cells exposed to palmitate ($p<0.05$). HAT activity was elevated 5-fold ($p=0.01$) in cells exposed to 0.5 mM palmitate compared with control cells while HDAC activity remained unaltered. This suggests that lipotoxicity induces epigenetic changes in the clonal beta-cells. Gene expression was analyzed after exposure to 0.5 mM palmitate. Four genes, three which are previously known to affect beta-cell function, were found to have altered expression on the mRNA level in lipotoxic beta-cells. These genes were also found to be epigenetically regulated by enrichment of the histone modifications H3Ac, H3K4me3 and H3K27me3. Data were obtained from 5 independent experiments and significance was evaluated using students t-test or Wilcoxon signed-rank test. Significance was assumed when $p<0.05$.

Conclusion: Lipotoxicity reduced glucose-stimulated insulin secretion in clonal beta-cells, probably due to metabolic defects. Elevations in HAT activity indicate that lipotoxicity induces epigenetic changes in the clonal beta-cells. Also, the expression and histone mark enrichment of four genes were found to be regulated by lipotoxicity.

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Involvement of p66Shc in FFA-mediated beta cell death

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Background and aims: The adaptor protein p66Shc mediates apoptosis and oxidative stress in multiple cell types. Chronically increased free fatty acid (FFA) levels induce beta-cell dysfunction and death, thus contributing to the pathogenesis of type 2 diabetes. The aim of this study was to investigate the potential regulatory effects of FFA on the p66Shc protein in rat insulinoma cells INS-1.

Materials and methods: Expression and phosphorylation levels of intracellular specific signaling molecules were assessed by immunoblotting techniques. Gene expression was evaluated by qRT-PCR. Beta-cell apoptosis was quantified by an ELISA assay evaluating oligosome release into the cytosol. Wild-type p66Shc and mutant p66Shc, in which Ser36 had been replaced by Ala (p66Shc-Ala36), were selectively overexpressed following infection with recombinant adenoviruses.

Results: Exposure of INS-1 cells to 0.5 mM palmitate induced a selective 2- to 3-fold increase in both mRNA and protein levels of p66Shc, evaluated by qRT-PCR and immunoblotting, respectively ($p<0.05$), without affecting the other Shc protein isoforms; furthermore, palmitate enhanced p66Shc phosphorylation on Ser36 ($p<0.05$). The effects on p66Shc were associated with a 2.5-fold increase in cell apoptosis, evaluated by measuring cytosolic oligosomes ($p<0.05$). When INS-1 cells were pretreated with pifithrin- α , an inhibitor of the tumor suppressor protein p53, the palmitate-induced increase in p66Shc expression was completely abrogated ($p<0.05$), suggesting the involvement of p53 in the FFA-p66Shc pathway. INS-1 cells with adenovirus-mediated overexpression of p66Shc (Ad/p66Shc) were next studied. Ad/p66Shc INS-1 cells showed increased rates of basal and palmitate-induced apoptosis, as compared with wild-type or mock-transduced cells ($p<0.05$). p66Shc overexpression was also associated with increased phosphorylation of p66Shc on Ser36, both under basal conditions and following exposure to palmitate ($p<0.05$). By contrast, overexpression of a phosphorylation-defective p66Shc protein, in which Ser36 had been mutated to Ala, did not enhance basal and reduced palmitate-induced apoptosis ($p<0.05$).

Conclusion: The FFA palmitate triggers the p53-mediated upregulation of the p66Shc protein in INS-1 cells. This is associated with increased cell apoptosis, which requires p66Shc phosphorylation on Ser36. Thus, p66Shc acts as a novel signaling intermediate in the FFA-mediated beta-cell damage, and may represent a potential therapeutic target to prevent the deleterious effects of lipotoxicity on pancreatic beta-cells.

PS 022 Stressed out islets: ER stress

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Zcchc12/Sizn1 is a novel ER stress-responsive gene expressed in the pancreatic beta cell

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Background and aims: Recent studies have shown decreased pancreatic β -cell mass to be a common feature of subjects with type 2 diabetes mellitus. Stress-mediated apoptosis is considered as one of the causes of β -cell loss. Especially, pancreatic β -cells are vulnerable to endoplasmic reticulum (ER) stress because of continuous and abundant production of insulin even in the physiological conditions. Therefore, we hypothesized that ER stress response or the unfolded protein response (UPR) in the β -cell could be different from that in other cell types, such as NIH3T3 fibroblasts.

Materials and methods: Microarray analysis was conducted employing RNA extracted from MIN6 β -cells and NIH3T3 fibroblasts exposed to 1.0 μ g/ml tunicamycin for 12 hours. Luciferase reporter assays were used to analyze promoter responsiveness to ER stress inducers. Stable transformants of MIN6 cells expressing shRNA against identified genes were created to explore their functions.

Results: There were many genes differentially expressed in response to the ER stress inducer between MIN6 and NIH3T3 cells. A gene expressed in MIN6 but not in NIH3T3 cells and induced more than two-fold was Zcchc12/Sizn1. Zcchc12 (zinc finger CCHC domain containing 12)/Sizn1 (Smad interacting zinc finger protein 1) is a recently identified transcriptional co-activator in the bone morphogenic protein signaling pathway. We confirmed by northern blot analysis that Zcchc12 mRNA was highly induced by thapsigargin and moderately by tunicamycin in MIN6 cells. We also found 2.5-fold increases in Zcchc12 mRNA levels in WFS1-knockout islets, which suffer from ER stress, as compared to wild-type islets. Northern blot analysis revealed that Zcchc12 is expressed in brain but not other tissues examined. The Zcchc12 gene contains 4 exons and the entire coding sequence is in the 4th exon. The upstream ~2 kbp fragment containing three introns was found to have promoter activity which was increased 2.7 ± 0.6 -fold by 0.5 μ M thapsigargin. The dominant-negative mutant of ATF4 suppressed thapsigargin-induced promoter activation by $72 \pm 14\%$. We also found that ZCCHC12 is located in the nucleus through double nuclear localization signals enriched in basic amino acid residues. Thapsigargin-induced expression of eif4E-binding protein 1, a UPR protein, was lower in MIN6 cell stable transformants expressing shRNA against Zcchc12 mRNA.

Conclusion: We identified the Zcchc12/Sizn1 gene as a novel ER-stress responsive gene expressed in the β -cell. Our data suggest that ZCCHC12 plays a role in modulation of the UPR in this cell type.

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BIRC proteins provide rheostat control of stress signalling in pancreatic beta cells

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Background and aims: TNF- α contributes to the pathogenesis of Type 1 diabetes by impairing pancreatic beta cell function and causing beta cell apoptosis. How TNF- α directs these processes remains to be elucidated. Baculoviral Inhibitor of Apoptosis Repeat Containing (BIRC) proteins are shown to be associated with the TNF signaling pathways and may play a role in regulating apoptosis. This study aims to elucidate the role of BIRC proteins in pancreatic beta cells.

Materials and methods: BIRC mRNA expression was analysed by microarray and quantitative RT-PCR. All transfection experiments were performed in MIN6 pancreatic beta cells. The BIRC3 proximal promoter and the BIRC3 expression construct were cloned according to reference sequences from GenBank. For cytokine treatment, MIN6 and primary mouse and human islets were stimulated with TNF- α (200 U/mL) for 4 h. To block transcription, cells were pre-treated with 1 μ M Actinomycin D for 1 h. In silico BIRC3 promoter analysis was conducted using PROMO 3.0. For in situ promoter analysis, transfected cells were measured by luciferase assay. To block nuclear factor- κ B (NF- κ B) activation, cells were pre-treated with 50 μ M PDTC for 1 h. Western blotting was employed to study NF- κ B signaling in primary islets

of knockout mice (BIRC2^{-/-} and BIRC3^{-/-} and BIRC2^{-/-}BIRC3^{-/-} (DKO) mice) by measuring phosphorylated and total I κ Ba levels.

Results: Following ligation of TNF receptor, we found BIRC3 but not BIRC1, 2, 4-6 to be induced in MIN6 cells and in primary mouse and human islets. Blockade of gene transcription prevented TNF- α -induced BIRC3 expression (>80%, $p < 0.01$). Analysis of the BIRC3 promoter revealed 3 putative NF- κ B and an AP-1 binding sites. The BIRC3 proximal promoter region was inducible by TNF- α (2-fold, $p < 0.05$). Thus NF- κ B is a major regulator of BIRC3 transcription. Over-expression of BIRC3 induced activation of an NF- κ B reporter (~2-fold, $p < 0.05$) in MIN6 cells. Further, BIRC3 potentiated TNF- α -induced NF- κ B activation by 8-fold, $p < 0.001$. Contrary to other cell types, BIRC3^{-/-} islets showed normal NF- κ B activation. Some data show BIRC2 is able to compensate for loss of BIRC3. Therefore DKO mice were generated. We found DKO islets were still able to activate TNF- α -mediated NF- κ B signaling. These data suggest that BIRC2 and BIRC3 are not essential for TNF- α -mediated NF- κ B signaling in islet cells. However, BIRC2 and BIRC3 did show unexpected phenotypes independent of NF- κ B. BIRC2^{-/-} islets exhibited increased basal mRNA expression of CCL2 and CXCL10 ($p < 0.01$), as well as increased TNF- α -stimulated ICAM-1 and CCL2 mRNA expression ($p < 0.001$). In contrast, BIRC3^{-/-} islets showed reduced TNF-stimulated CCL2 and CXCL10 mRNA expression ($p < 0.01$).

Conclusion: Our data highlights the complex roles BIRC proteins play in islet stress signaling. On the one hand BIRC3 potentiates NF- κ B when increased but on the other BIRC3 is not necessary per se for TNF- α activation of NF- κ B. Further, in the absence of BIRC2 or BIRC3, expression of CCL2, CXCL10 and ICAM-1 are dysregulated. These data present the novel insight that BIRC2 and BIRC3 provide rheostat control of inflammatory pathways in islet cells.

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Implication of SERCA3 Ca²⁺ channel in protective losartan effects from glucotoxic ER and oxidative stress on human pancreatic islets and MIN6B1 beta cells

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Background and aims: Chronic high glucose concentrations increases glucose metabolism through oxidative phosphorylation. This causes mitochondrial dysfunction and excess of reactive oxygen species (ROS) in beta-cells, due to their low levels of ROS-detoxifying enzymes. Beta-cells present a developed ER in order to answer to high demand for synthesis of insulin. Recent data suggest that ER stress is present in human beta-cells and that this could be a common mechanism for the two major pathophysiological events in type 2 diabetes: insulin resistance and beta-cell failure. Prospective studies show a significant reduction in the risk of type 2 diabetes after blockade of the renin angiotensin system (RAS). Since RAS has been found in pancreatic islets, we hypothesized that these beneficial effects could be attributed to direct actions on islets. Our present study evaluates the effects of the angiotensin II receptor blocker Losartan on stress induced by glucose, and its potential implication on specific ER Ca²⁺ release channels, in human islets and in murine MIN6B1 beta-cells.

Materials and methods: Human islets from 8 distinct donors and MIN6B1 cells were studied following culture in presence of 5.5 mmol/l and 16.7 mmol/l glucose concentrations with or without 5 μ mol/l Losartan during the last 48 hrs. ER stress-related mRNA, Caspase-3, and Serca3 mRNA were detected by real-time RT-PCR, insulin secretion by IRMA. ROS levels were determined by measuring DCF oxidation. Mitochondrial human beta-cell morphology was analysed by electron microscopy.

Results: In human islets, chronic high glucose exposure reduced acute glucose-induced insulin secretion by 50% compared to 5.5 mmol/l glucose ($p < 0.05$). Losartan restored this response ($p < 0.05$). Chronic exposure to glucose up-regulated ROS levels by 60% ($p = 0.02$), and reversed by half with Losartan. Quantitative electron microscopy analysis revealed swollen beta-cell mitochondria with high glucose condition (size: 701 ± 144 vs. 405 ± 100 ; $p < 0.0001$), with inner membrane disruption and abnormal cristae. Losartan dramatically improved mitochondrial morphology and reduced mitochondrial size (701 ± 144 vs. 405 ± 143 ; $p < 0.0001$). GRP78, spliced XBP-1, and ATF4 mRNA expressions increased with high glucose concentration ($x_{2.2} p < 0.01$, $x_{1.5} p < 0.009$, $x_{2} p < 0.05$ respectively). The expression of CHOP, an ER stress marker of apoptosis, remained the same, but caspase-3 mRNA

clearly increased ($x2.2$; $p=0.03$). Addition of Losartan to 16.7 mmol/l glucose medium reduced significantly ER stress marker's expression: GRP78 mRNA by 55% ($p<0.01$), XBP-1s mRNA by 51% ($p<0.02$), ATF4 by 54% ($p<0.05$), and apoptose marker Caspase-3 mRNA by 43% ($p=0.02$) as compared to high glucose condition alone. Chronic exposure to glucose up-regulated Serca3 mRNA expression by 180% ($p=0.026$) and Losartan reversed this effect by 33% ($p=0.04$). In MIN6B1 cells, GRP78, spliced XBP-1, CHOP, and ATF4, Serca3 were increased in high glucose condition ($x1.3$, $x8.3$, $x1.3$, $x2.3$, $x1.3$ respectively). Losartan reversed these effects.

Conclusion: Chronic high glucose exposure of human islets and murine beta-cells increases oxidative and ER stress. Blockade of RAS protects them against ER and oxidative stress, prolongs beta-cell integrity, and modulates ER Ca^{2+} release channels. These findings may have important therapeutic consequences both for prevention of type 2 diabetes and islet transplantation. *Supported by: Centaure*

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The role of the ubiquitin-proteasome system in type 2 diabetes beta cell dysfunction

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Background and aims: Beta cell failure plays a fundamental role in the onset and progression of type 2 diabetes (T2D). Recent evidences in cell lines and rodent models suggest that the ubiquitin-proteasome system (UPS), a major intracellular degradative system, may be involved in the regulation of beta cell function and turnover. Limited information is however available on the UPS in beta cells of human T2D subjects.

Materials and methods: We studied islet preparations from 16 T2D and 25 Ctrl individuals with similar clinical characteristics. Gene expression was evaluated by microarray analysis (Affymetrix Human HG U133A) and quantitative RT-PCR of selected genes. Ubiquitinated proteins were assessed by immunohistochemistry and proteasome activity (trypsin-like) was measured by a luminescent assay. The direct effect of a proteasome inhibitor (MG132), 0.5 mmol/l palmitate and 2.4 µg/ml metformin was also assessed.

Results: Microarray analysis showed that 63 genes involved in the UPS were differently expressed in T2D islets (4 upregulated, 59 down regulated). When quantitative RT-PCR experiments of selected genes were performed significant upregulation of SAE-1 (of the ubiquitin-activating enzyme family E1) and significant downregulation of UBE2K (of the ubiquitin-conjugating enzyme family E2), UCHL1 (a de-ubiquitinating enzyme) and PSMB7 (proteasomal subunit with trypsin-like activity) were demonstrated in T2D samples. Immunohistochemistry revealed the presence of ubiquitinated proteins in beta cells, and proteasome activity was reduced in the diabetic islets (4364±1975 vs 8748±4238 RLU/ng DNA, $p<0.02$). MG132 suppressed proteasome activity of Ctrl HI by 80–90% ($p<0.01$), which was associated with lower glucose-stimulated insulin secretion (45±7% of untreated islets, $p<0.01$). In addition, 24 h exposure to palmitate reduced proteasome activity by 20%, which again was related with lower insulin release. T2D islets pre-exposed (24 h) to metformin showed increased proteasome activity (+35%), which was accompanied by improved glucose-stimulated insulin secretion.

Conclusion: These results show the importance of UPS alterations as a possible mechanism associated to beta cell dysfunction in human T2D, and propose that some of these changes may be corrected pharmacologically.

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Metabolic stress-induced beta cell failure contributes to diabetes in Friedreich's ataxia

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Background and aims: Friedreich's ataxia (FA) is an autosomal recessive neurodegenerative disease caused by a GAA trinucleotide repeat expansion

in the first intron of the frataxin (Fxn) gene leading to a 60–90 % reduction in the mitochondrial protein Fxn. FA patients have a high prevalence of impaired glucose tolerance (IGT) and diabetes. Our aims were to study the relative contribution of insulin resistance and beta-cell failure and the pathogenic mechanisms involved in FA diabetes.

Materials and methods: 41 FA patients and 53 controls underwent oral and IV glucose tolerance tests (GTT). Insulin sensitivity index (SI) and acute insulin response to glucose (AIRg) were used to calculate the disposition index (DI). In vitro and in vivo disease models were used to study the impact of Fxn deficiency on beta-cell function and survival. Male Fxn-deficient mice (KIKO) underwent IP insulin (ITT) and GTT after 3 and 6 months on regular (RD) or high fat diet (HFD). KIKO and wild type (WT) beta-cells were examined by electron microscopy (EM). Fxn was knocked down by siRNA (siFxn) in clonal INS-1E cells, primary rat beta-cells and dispersed human islets. Beta-cell apoptosis was examined by Hoechst 33342/propidium iodide staining 24h after exposure to oleate (0.5 mmol/l, OL) or the endoplasmic reticulum (ER) stressors cyclopiazonic acid (25 µmol/l, CPA), tunicamycin (5 µg/ml, TU) or brefeldin A (0.1 µg/ml, BR), alone or in combination with the adenylate cyclase stimulator forskolin (20 µmol/l, FK).

Results: 50% of the FA patients had IGT and 10% diabetes, compared to 29% IGT and no diabetes in controls. FA patients were insulin resistant (SI 17 ± 2 vs $25\pm2 \times 10^{-5} \text{ min}^{-1}/(\mu\text{U/ml})$, $p<0.01$) and this was not compensated for by increased AIRg, resulting in a markedly reduced DI (FA 862 ± 156 vs controls $1507\pm169 \times 10^{-5} \text{ min}^{-1}$, $p<0.01$) indicating beta-cell failure. KIKO mice were also insulin resistant (ITT after 3 months on RD: area above glycemic curve 37 ± 8 vs 66 ± 6 in WT, $p<0.05$), and developed impaired beta-cell function after 6 months on HFD. Beta-cells from KIKO mice had clear ER dilation, as evaluated by EM, indicating ER stress. Fxn knockdown increased apoptosis in rat beta-cells ($10\pm1\%$ apoptosis for siFxn vs $8\pm1\%$ for control siRNA (siCT), $n=6$, $p<0.05$) and dispersed human islets ($27\pm1\%$ for siFxn vs $16\pm2\%$ for siCT, $n=4$, $p<0.01$). Fxn knockdown also sensitized INS-1E cells to OL- and ER stress-induced apoptosis (OL $20\pm2\%$ for siFxn vs $13\pm3\%$ for siCT, $n=6$, $p<0.01$; CPA $48\pm2\%$, TUN $25\pm3\%$, BR $37\pm2\%$ apoptosis for siFxn vs, respectively, $38\pm3\%$, $19\pm2\%$, and $22\pm4\%$ for siCT, $n=4$, $p<0.05$). FK treatment partially prevented OL- and ER stress-induced apoptosis in Fxn-deficient beta-cells (OL+FK $13\pm4\%$ apoptosis vs OL $19\pm4\%$, $n=8$, $p<0.01$; TUN+FK $9\pm2\%$ apoptosis vs TUN $27\pm2\%$, $n=3$, $p<0.01$).

Conclusion: Beta-cell dysfunction plays a key role for IGT and diabetes in FA patients. In line with these clinical findings, KIKO mice develop beta-cell failure after high fat feeding. This may be due to metabolic stress-induced beta-cell apoptosis, a process that seems to be mediated by ER stress. Inducers of cAMP have a protective role and may have therapeutic potential.

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GLP-1 exerts a protective role in pancreatic beta cells with WFS1-deficiency

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Background and aims: Recent development of incretin-based drugs demonstrates promising outcomes for treatment of type 2 diabetes. However, mechanisms whereby incretin hormones exert their effects on pancreatic β -cell failure are not fully understood. We investigated whether GLP-1 improves glucose homeostasis and β -cell failure under endoplasmic reticulum (ER) stress, which is now accepted as one of the causes of β -cell failure, commonly observed in type 2 diabetes.

Materials and methods: MIN6 cell viability was analyzed under the treatment with pharmacological ER stress inducers, thapsigargin (ER calcium pump inhibitor) and tunicamycin (N-glycosylation inhibitor) in the presence or absence of GLP-1 (100 nM) for 36 hours. In addition, mRNAs of several ER stress-related proteins and apoptosis-related proteins were analyzed by a semi-quantitative PCR analysis in MIN6 cells exposed to thapsigargin in the presence or absence of GLP-1. To further clarify the protective role of GLP-1 under ER stress in β -cells, vildagliptin (50 mg/kg), a DPP-4 inhibitor, was orally administrated twice a day to WFS1-knockout ($Wfs1^{-/-}$) mice, a mouse model of genetically defined human diabetes caused by ER stress in β -cells.

Results: GLP-1 increased cellular viability under ER stress induced by acute treatment with thapsigargin or tunicamycin in MIN6WT cells. We also found that thapsigargin-mediated induction of caspase-3 mRNA, a hallmark of apoptosis, was suppressed by GLP-1. Treatment of GLP-1 significantly sup-

pressed thapsigargin-induced mRNA levels of spliced XBP-1 and ATF4 with tendency toward reduction in GRP78 mRNA expression in MIN6WT cells. Wfs1^{-/-} mice at 9 weeks of age, which were given vildagliptin for 4 weeks, showed no change in blood glucose as compared with vehicle-treated Wfs1^{-/-} mice. However, an intraperitoneal glucose tolerance test showed improved glucose tolerance in vildagliptin-treated Wfs1^{-/-} mice with increased glucose responsiveness of insulin secretion. Moreover, vildagliptin-treatment significantly increased pancreatic insulin contents by 32% in Wfs1^{-/-} mice, and the effect was blocked by additional administration of the GLP-1 antagonist, exendin (9-39). Electronmicroscopic studies revealed that vildagliptin reduced cell numbers of β -cells with swollen ER in Wfs1^{-/-} mice.

Conclusion: The results in vitro suggested that GLP-1 ameliorated ER stress, resulting in less induction of three pathways of the UPR and improved cellular viability. Moreover, the protective role of GLP-1 against ER stress on β -cell was confirmed in Wfs1^{-/-} mice. Recent studies indicated sequence variants in WFS1 gene confer risk of type 2 diabetes. Thus, our results strengthen the mechanistic rational of using this drug for the treatment of type 2 diabetes.

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Glucose-induced changes in mitochondrial redox status in rat pancreatic beta cells

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Background and aims: Measuring reactive oxygen species (ROS) in living cells often relies on the use of fluorescent probes (hydroethidine, dichlorodihydrofluorescein). Besides their photosensitivity and low selectivity, these tools do not allow studying ROS production in subcellular compartments. Using new redox-sensitive ratiometric fluorescent proteins (FP) could overcome these technical limitations. HyPer, derived from OxyR and circularly permuted Yellow FP, is sensitive to H₂O₂. roGFPs, derived from Green FP, monitor changes in thiol/disulfide ratio (e.g. the GSH/GSSG ratio). Possible pH sensitivity of these probes may prevent their use in beta cells, for cytosolic and mitochondrial pH are affected by nutrients. In this study, we tested the adequacy of mitochondria targeted HyPer (mt-HyPer) and roGFP1 (mt-roGFP) to monitor glucose-induced changes in mitochondrial redox status in rat beta cells.

Materials and methods: Rat islet cell clusters were infected with an adenovirus coding mt-HyPer or mt-roGFP (CMV promoter driven), or the mitochondrial pH sensor mtAlpHi (under the control of the rat insulin promoter). Cell clusters were perfused with Krebs buffer (pH 7.4) containing 1 mg/ml BSA and gassed with O₂:CO₂ (94:6). Fluorescence at 535 nm was measured every 30s during excitation at 480/400 nm for mt-roGFP, 420/495 nm for mt-HyPer and 490 nm for mtAlpHi.

Results: Two days after adenoviral infection, mt-HyPer and mt-roGFP were expressed and correctly localized in the mitochondria of most beta cells within clusters. During perfusion with 10 mM glucose, mt-HyPer and mt-roGFP fluorescence ratios were stable. As expected, addition of 100 μ M H₂O₂ rapidly increased both ratios 2 to 3-fold, attesting the ability of these probes to sense H₂O₂. Unfortunately, mt-HyPer was highly pH sensitive: its fluorescence ratio rapidly decreased upon acidification with 30 mM Na⁺-acetate and increased upon alkalinisation with 30 mM NH₄Cl. Consistently, mitochondrial metabolism affected mt-HyPer and mtAlpHi signals similarly: an increase upon acute glucose stimulation from 2 to 10 mM that plateaued between 10 and 30 mM glucose, a transient increase followed by a slow decrease upon glucose reduction from 10 to 2 mM, and a rapid and reversible decrease upon addition of 3 mM azide to 10 mM glucose. In contrast, mt-roGFP fluorescence ratio was not affected by NH₄Cl or azide and responded differently upon changes in glucose metabolism. Thus, mt-roGFP signal increased upon glucose reduction from 10 to 2 mM and decreased upon return to 10 mM glucose. The signal was unaffected by a rise in glucose from 10 to 30 mM. Importantly, the increase by low glucose was abrogated by the catalytic antioxidant Mn(III) tetrakis(4-benzoic acid) porphyrin (50 μ M TBAP).

Conclusion: mt-HyPer fluorescence ratio is highly pH-sensitive, thereby preventing its use as a reliable indicator of glucose-induced changes in mitochondrial H₂O₂ in beta cells. In contrast, mt-roGFP1 fluorescence ratio is pH-insensitive and adequately monitors changes in mitochondrial redox status. Our results also show that low glucose rather than high glucose triggers mitochondrial oxidative stress in rat beta cells.

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Wfs1 deficiency impairs glucose metabolism and induces XBP1(s) gene expression levels in mice with a diabetes related phenotype

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Background and aims: Wfs1 gene encodes for an endoplasmic reticulum stress (ER) membrane protein and its deficiency causes Wolfram syndrome, which is associated with diabetes mellitus and optical atrophy. ER stress plays an important role in the pathogenesis of diabetes, contributing to β -cell loss and insulin resistance. The X-box binding protein 1 (XBP1) is a transcription factor and a key regulator in the unfolded protein response (UPR). The aim of the present study was to evaluate the impact of Wfs1 deficiency on hyperglycaemia in mice and to determine the relative expression levels of XBP1 spliced (s) gene in liver and kidney tissue.

Materials and methods: Wfs1 deficient mice were generated by invalidating the 8th exon of the Wfs1 gene. The study included 23 male mice [(129S6/SvEvTac \times 129S6/SvEvTac) \times (129S6/SvEvTac \times 129S6/SvEvTac)] assigned to three subgroups: Wfs1^{+/+} (WT, n=5), Wfs1^{+/-} (HZ, n=9) and Wfs1^{-/-} (KO, n=9). Breeding and genotype analysis of mice were carried out in the Department of Physiology, University of Tartu. Insulin plasma measurements, intraperitoneal glucose tolerance test (ipGTT) (2g/Kg) following fasting for 14 hours, non-fasting blood glucose and the XBP1 spliced gene expression levels in liver and kidney tissues have been analyzed. Data analysis performed using the Wilcoxon test and linear regression analysis was applied where appropriate. A p value of <0.05 was considered significant for all analyses.

Results: The ipGTT showed that the blood glucose levels on 60 min after glucose administration, were significantly higher in Wfs1KO mice when compared to Wfs1HZ (p=0.0039) and Wfs1WT (p=0.041) mice, respectively. The non-fasting glucose levels also found to be significantly higher in Wfs1KO mice when compared to Wfs1HZ (p=0.0046) and Wfs1WT (p=0.027) mice, respectively. The insulin plasma levels found to be significantly higher in the group of Wfs1HZ mice when compared to the group of Wfs1KO (p=0.00008) and Wfs1WT (p=0.041) mice, respectively. The expression of the XBP1(s) gene in liver tissue was found to be significantly higher in both the Wfs1HZ (OR=1.06, p=0.024) and Wfs1KO (OR=1.06, p=0.22) group when compared to the Wfs1WT group. Moreover, the XBP1(s) gene expression analysis in kidney tissue showed that the XBP1(s) levels were significantly higher in the group of Wfs1HZ mice (OR=1.08, p=0.012) in comparison to the Wfs1WT group.

Conclusion: Wfs1 deficiency increases blood glucose levels in Wfs1KO mice and induces the expression of XBP1(s) gene in liver tissue. A better knowledge on the protein homeostasis regulated by the ER signaling pathways and the Wfs1 gene with their interactions to β -cell biology will have a direct impact on future therapies for diabetes.

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Characterisation of three novel human insulin-releasing pancreatic beta cell lines produced by electrofusion

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Background and aims: Three novel human insulin releasing cell lines designated 1.1B4, 1.4E7 and 1.1E7 were generated by electrofusion of freshly isolated of human pancreatic beta cells and the immortal human PANC-1 epithelial cell line. This study aimed to characterize the in vitro and in vivo function of these 3 novel human beta cell lines.

Materials and methods: Dispersed human islet cells and PANC-1 cells were electrofused and hybrid colonies were selected and cloned for high insulin output. Insulin secretion in acute 60min test incubations and insulin content was measured by radioimmunoassay. Expression of key human beta cell genes and proteins were examined by RTPCR, Western blot and immunofluorescence. Measurements were made of enzyme activity, metabolism of glucose by radioactive scintillation counting and FLEXstation™ analysis of $[Ca^{2+}]_i$. In vivo analysis was carried out after subcapsular implantation of the 1.1B4 cell line in streptozotocin-diabetic immunocompromised SCID mice.

Results: 1.1B4 cells exhibited a stepwise 2.3-fold insulin-secretory response over the range 0–16.7mM glucose. Both 1.4E7 and 1.1E7 cells showed a maximal 1.6 and 1.5-fold secretory responses to 5.6mM and 11.1mM glucose respectively. Inclusion of 200uM IBMX significantly increased secretory responses by 20–100% ($p < 0.001$). Leucine, alanine, arginine (all 20mM), tolbutamide (200uM), glibenclamide (100uM), GLP-1, GIP or CCK-8 (all 10^{-9} M) significantly stimulated insulin release by 1.1–1.4 fold in each of the 3 cell lines ($p < 0.05$ – $p < 0.001$). Inhibitors, 400uM diazoxide, 20uM verapamil or Ca^{2+} chelation with EGTA significantly reduced insulin release from the cell lines by 20–50% ($p < 0.01$ – $p < 0.001$), 20–40% ($p < 0.05$ – $p < 0.001$) and 20–40% ($p < 0.01$ – $p < 0.001$) respectively. Cellular insulin content at passage 17 was measured as 3.5 ± 0.2 , 3.6 ± 0.2 and 3.9 ± 0.2 ng/ 10^6 cells, for 1.1E7, 1.4E7 and 1.1B4 respectively. Immunohistochemistry revealed the presence of human specific insulin, proinsulin and C-peptide in 1.1B4 cells and intact human proinsulin was detected in all 3 beta cell lines with proinsulin:insulin ratios of approximately 1:20. Western blot, RT-PCR and immunohistochemistry showed expression of the major genes involved in proinsulin processing and the pancreatic beta cell stimulus-secretion pathway including PC1/3, PC2, GLUT-1, glucokinase, K-ATP channel complex (Sur1 and Kir6.2) and the voltage-dependent L-type Ca^{2+} channel. Analysis of metabolic function showed that all 3 cell lines exhibited significantly higher ($p < 0.01$ – $p < 0.001$) glucokinase and lower ($p < 0.01$ – $p < 0.001$) hexokinase activity compared to parental PANC-1 cells with 1.1B4 and 1.4E7 showing significant increases ($p < 0.05$) in glucose oxidation rates at 1.1–16.7mM glucose compared to parental PANC-1. Acute exposure to 16.7mM glucose, 20mM alanine or depolarizing concentrations of 30mM K^+ were all able to significantly increase $[Ca^{2+}]_i$ ($p < 0.001$, $p < 0.05$ – $p < 0.001$ and $p < 0.001$, respectively). Streptozotocin-diabetic SCID mice implanted subcapsularly with 5×10^6 1.1B4 cells exhibited time-dependent decrease of hyperglycaemia and improved glucose tolerance.

Conclusion: These novel cell lines exhibit stable characteristics reminiscent of normal beta cells, thereby providing an unlimited source of human insulin producing cells for biochemical studies and pharmacological drug testing

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Bile acids affect the function of murine pancreatic beta cells

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Background and aims: Bile acids (BAs) play an important role in digestion and cholesterol metabolism but also function as signalling molecules in several metabolic pathways. In the liver BAs affect insulin action and interfere with glucose homeostasis. The aim of the study was to test whether BAs influence stimulus-secretion coupling in pancreatic beta-cells.

Materials and methods: Ion currents and the membrane potential (V_m) of mouse beta-cells were measured with the patch-clamp technique, cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) by fura-2 and insulin secretion by RIA.

Results: Taurochenodeoxycholic acid (TCDC) increased $[Ca^{2+}]_i$ ($n=9$, $p < 0.05$) in physiological concentrations (500 nM) due to Ca^{2+} influx through L-type Ca^{2+} channels which resulted in enhanced glucose-induced insulin secretion (GIIS) ($n=7$, $p < 0.05$). The stimulatory action of TCDC was due to changes in glucose-induced electrical activity. The fraction of plateau phase (FOPP), i.e. the percentage of time with spike activity, increased from 27.3 ± 4.4 % to 44.3 ± 6.0 % in the presence of 5 μ M TCDC ($n=7$, $p < 0.01$). At a threshold glucose concentration (6 mM) TCDC (10 μ M) depolarized V_m and action potentials appeared ($n=8$, $p < 0.001$). The underlying depolarisation was evoked by closure of K_{ATP} channels ($n=9$, $p < 0.001$). However, in excised inside/out patches TCDC had no effect ($n=11$). Therefore, TCDC-induced depolarization seems to require cytosolic components. It is known that BAs interact with several receptors including the nuclear farnesoid X receptor (FXR) that is involved in the regulation of glucose and energy homeostasis. The specific FXR agonist GW4064 mimicked the effects of TCDC on stimulus-secretion coupling in the same concentration range. GW4064 depolarized V_m ($n=5$, $p < 0.01$), triggered Ca^{2+} influx and enhanced GIIS ($n=10$, $p < 0.05$). In contrast, ursodeoxycholate (UDC) that had a much lower affinity to FXR than TCDC did not change $[Ca^{2+}]_i$ ($n=7$). Islets of FXR-KO mice did not show any increase in insulin secretion with TCDC ($n=6$). Likewise, all effects could be suppressed by the FXR antagonist guggulsterone. These results indicate that FXR activation is indispensable for the stimulatory effects of TCDC. In islets of SUR1-KO mice lacking functional K_{ATP} channels TCDC did not alter GIIS ($n=8$) suggesting that FXR activation influences K_{ATP} channel activity.

Conclusion: The results provide evidence for a novel link between FXR activation and inhibition of K_{ATP} channels. BA signalling in beta-cells may constitute a further connection between food intake and the control of insulin secretion and may give rise to a new pharmacological approach to interfere with beta-cell stimulus-secretion coupling.

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The bile acids TGR5 receptor regulates insulin secretion through stimulation of pancreatic δ -cells somatostatin release

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Background and aims: The bile acids G protein-coupled receptor TGR5 controls energy expenditure in brown adipose tissue and skeletal muscle as well as GLP-1 secretion in the gastro-intestinal tract by L-cells. We addressed whether TGR5 is expressed in human pancreatic islets and whether it is involved in insulin secretory function.

Materials and methods: Experiments were realized on human pancreata and on isolated islet of Langerhans ($n=12$, mean age= 53.3 ± 4.0), pancreas were procured from brain-dead non-diabetic ($n=8$, mean HbA1C= 5.7 ± 0.1) and diabetic ($n=4$, mean HbA1C= 8.2 ± 1.1). TGR5 expression was analyzed by immunohistochemistry, western-blot and QPCR. Functional parameters were studied (insulin content, acute insulin release in response to glucose stimulation, somatostatin release) on isolated islets in presence or absence of 3-(2-Chlorophenyl)-N-(4-Chlorophenyl)-N,5-dimethylisoxazole-4-carboxamide a specific TGR5 agonist.

Results: TGR5 was detected in pancreatic islets and localized specifically on δ -cells. In addition, we found a three fold higher expression of TGR5 in diabetic islets correlated with an increased number of δ -cells (30%). Basal insulin was not affected by addition of TGR5 agonist (50 μ M) to human islets culture medium with 2.8mmol/l glucose concentration. However, we show an increase (25%) in somatostatin release and potent inhibition of Glucose Stimulated-Insulin secretion (20mmol/l glucose concentration) led to significant decreases (30%) of stimulated insulin release.

Conclusion: TGR5 is a new player in the control of the Glucose Stimulated-Insulin release through control of somatostatin release. Altogether, these data identify a new role for bile acids and TGR5 in the regulation of glucose homeostasis directly in pancreatic islet cells.

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Insulin stimulation leads to oscillatory interactions between insulin receptor B-type and PI3K-C2α or Shc in pancreatic beta cells

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Background and aims: It has been shown that stimulation of glucokinase and c-fos gene transcription by insulin in pancreatic beta cells requires the activation of the insulin receptor B-type (IR-B) and signaling through PI-3-kinase C2α (C2α) or Shc respectively. The activation of c-fos gene transcription is dependent on clathrin mediated IR-B-endocytosis and on the C-terminal YTHM-motif, while glucokinase gene activation is generated from membrane standing IR-B requiring an intact juxtamembrane NPEY-motif. However it remains unclear whether and how these two signaling cascades are interrelated. Temporal analysis of the interaction of IR-B with either Shc or C2α after insulin stimulation by FRET-analysis in combination with mutations in the insulin receptor is used as a tool to investigate this question.

Materials and methods: MIN6 cells were grown on coverglasses and transfected with IR-B-Venus or its respective mutants and CFP- C2α or CFP-Shc. 48h post transfection, cells were pre-incubated in medium containing 2 mM glucose for 6 h and stimulated with 5mU/ml insulin for up to 10 min. Cells were fixed with 3% PFA in PBS in 1 min intervals post stimulation. FRET-analysis by acceptor photobleaching using confocal microscopy was performed for 10 cells per timepoint and condition. FRET-efficiency (Ef) was calculated by the following formula: (CFP-Intensity after bleaching - CFP-intensity before bleaching)/CFP-intensity before bleaching.

Results: Interaction of IR-B with either C2α or Shc changed after insulin stimulation in an oscillatory manner. Interaction of IR-B and Shc started with a high Ef of 0.213 ± 0.023 and oscillated with a period of 4 min (minimum -0.009 ± 0.021 at 10 min, maximum 0.259 ± 0.023 at 4 min). In contrast, IR-B and C2α interaction started with a low Ef of 0.046 ± 0.016 . FRET efficiency here also oscillated with a period of 4 min (minimum -0.035 ± 0.013 at 8 min, maximum 0.225 ± 0.23 at 10 min), but phase-shifted by 0.5 periods towards the oscillations of the interaction of IR-B and Shc. Mutation of the juxtamembrane NPEY motif to NPEA abolished both interaction of C2α with IR-B and the changes triggered by insulin. It lead to an increase in Ef for IR-B-Shc interaction (0.465 ± 0.031 unstimulated) and slower oscillations after stimulation (period 6 min, minimum 0.288 ± 0.012 , maximum 0.492 ± 0.033) on a higher baseline. Mutation of the C-terminal YTHM motif to FTHM abolished Shc-IR-B interaction. The oscillatory interaction between this mutant and C2α was still present (starting at 0.059 ± 0.022), albeit slower (Period 6 min, minimum 0.059 ± 0.022 at 0 min, maximum 0.238 ± 0.022 at 3 min).

Conclusion: Interaction between IR-B and C2α or Shc after insulin stimulation occurs in an oscillatory manner. The oscillations of IR-B/ C2α and IR-B/ Shc interaction are antiparallel towards each other suggesting that the same receptor might activate both signaling cascades sequentially. Mutation of the two motifs YTHM or NPEY abolishes the interaction of either Shc or C2α respectively, but does not lead to an abolishment of oscillation of the interaction of IR-B with the other protein per se, but to change in period and amplitude of oscillation. These data suggest that binding of either Shc or C2α is not a pre-requisite of IR-B interaction with the other binding partner.

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Tigerinin-1R from the skin secretions of Vietnamese frog *hoplobatrachus rugulosus* exerts potent insulinotropic effects both *in vitro* and *in vivo*Y.H.A. Abdel-Wahab¹, O.O. Ojo¹, P.R. Flatt¹, J. Michael Conlon²;¹School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland, UK, ²Department of Biochemistry, Faculty of Medicine and Health Sciences, Al-Ain, United Arab Emirates.

Background and aims: Frog skin represents a valuable source of biologically active peptides that have potential for development into therapeutic agents. Studies have shown that several peptides from frog skin, first identified on the basis of antimicrobial activity, stimulate the release of insulin from pancreatic beta cells at non-cytotoxic concentrations. This study investigated the effects of a 12 amino-acid-residue cyclic, C-terminally α-amidated peptide termed tigerinin-1R (Arg-Val-Cys-Ser-Ala-Ile-Pro- Leu -Pro-Ile-Cys-His. NH₂), isolated from an extract of the skin of the Vietnamese frog *Hoplo-*

batrachus rugulosus (Dicroglossidae), on insulin secretion both *in vitro* and *in vivo*.

Materials and methods: Insulin-releasing effects of tigerinin-1R were studied using the glucose-responsive clonal pancreatic cell line, BRIN-BD11, while effects on glucose tolerance were investigated in high fat fed insulin resistant Swiss TO mice. Acute insulin-release studies were performed in Krebs Ringer bicarbonate buffer supplemented with 5.6mM or 16.7mM glucose in the absence (control) or presence of the synthetic forms of the peptide (0 - 3μM) and various known modulators of insulin secretion. Insulin release was measured by radioimmunoassay while membrane potential and intracellular calcium ([Ca²⁺]) were both determined by fluorometric assay using FLEXstationTM.

Results: At 5.6mM glucose, tigerinin-1R stimulated insulin release by 138% at 0.01nM ($P < 0.01$) with a maximum response at 3μM of 410 % above basal (4.10 ± 0.40 ng/10⁶ cells/20min; $P < 0.001$). At 16.7mM glucose, tigerinin-1R stimulated insulin secretion by 1.5-3.0-fold over the concentration range 0.03nM - 3μM ($P < 0.05$, $P < 0.001$ respectively). Tigerinin-1R (1μM)-induced insulin release was also enhanced ($P < 0.001$) by 200μM IBMX (2.0-fold at 5.6mM glucose) and 200μM tolbutamide (3.2-fold). At 16.7mM glucose in the presence of 30mM KCl, tigerinin-1R further enhanced ($P < 0.001$) insulin release (1.4-fold). Chelation of extracellular calcium caused a 12 % reduction ($P < 0.001$) in stimulatory effects of tigerinin-1R, whereas 50 μM verapamil decreased insulin release by 26% ($P < 0.001$). At 5.6mM glucose, 1μM tigerinin-1R induced membrane depolarization and significantly increased intracellular calcium (by 3.0 and 2.5-fold, respectively; $P < 0.001$). C-terminal amidation of tigerinin-1R was necessary for effective insulin releasing activity. The free acid form of tigerinin-1R was 10-fold less potent and produced a much lower maximum response (188 % of basal rate, $P < 0.001$). *In vivo*, intra-peritoneal administration of tigerinin-1R (75 nmol/kg body weight) together with 18mmol/kg glucose significantly ($P < 0.05$) enhanced insulin-release (1.7-fold) and improved glucose tolerance by 62% ($n=6$, $P < 0.05$) in mice with diet-induced insulin resistance. *In vitro* tests also showed that tigerinin-1R lacked antimicrobial activity and was non-hemolytic at concentrations up to 400μM.

Conclusion: Tigerinin-1R exerts strong insulinotropic effects on pancreatic beta cells both *in vitro* and *in vivo*. The glucose lowering action of tigerinin-1R encourages further investigation of its longer-term effects in type-2 diabetes.

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Chronic high glucose and pyruvate levels differentially effect beta cell functionI. Göhring¹, S. Malmgren¹, V.V. Sharoyko¹, D. Nicholls^{1,2}, H. Mulder¹;¹Unit of Molecular Metabolism, Lund University Diabetes Centre, Malmö, Sweden, ²Buck Institute for Age Research, Novato, USA.

Background and aims: Glucose-stimulated insulin secretion is coupled to increased cellular metabolic activity and an increase of the ATP/ADP ratio, leading to plasma membrane depolarization and exocytosis. Prolonged elevated glucose levels are, however, known to impair beta cell function and have been associated to both cytoplasmic and mitochondrial disturbances in glucose metabolism. We investigated whether overstimulation of pancreatic beta cells with high pyruvate is as deleterious as high glucose. This was thought to provide a better understanding of whether triggering factors of glucotoxicity are mainly of mitochondrial or cytoplasmic origin.

Materials and methods: INS-1 832/13 cells were cultured in either 2.8 mM glucose, 16.7 mM glucose or 2.8 mM glucose + 33.4 mM pyruvate for 48 h. Glucose-stimulated insulin secretion and glucose utilization were determined by radioimmunoassay and scintillation counting, respectively. Intracellular metabolite alterations were determined by gas chromatography-mass spectrometry. Plasma and mitochondrial membrane potential changes were determined in real-time, using laser scanning microscopy.

Results: After a 48 h culture in 16.7 mM glucose, glucose-stimulated insulin secretion was significantly suppressed ($p < 0.05$; 28.4 ± 3.3 ng/mg/ml) in comparison to cells cultured at 2.8 mM glucose (83.1 ± 4.2 ng/mg/ml) for 48 h. In addition, the insulin content was decreased by 92%. Furthermore, the mitochondrial membrane hyperpolarization response was greatly impaired. Moreover, the plasma membrane potential response was significantly suppressed (-71.3 ± 0.8 mV) in comparison to control cells, where raising glucose from 2.8 mM to 16.7 mM increased the potential from -80 ± 0.5 mV to -58 ± 4.7 mV. Glucose utilization was increased 1.3 fold by the chronic glucose culture at 16.7 mM. Several glycolytic and mitochondrial metabolites, including pyruvate, lactate and alpha-ketoglutarate, were greatly elevated in the chronic high glucose-treated cells. In contrast, cells cultured for 48 h at 2.8 mM glc +

33.4 mM pyruvate maintained their acute glucose-stimulated insulin secretory response (86.7 ± 8.9 ng/mg/ml), and even released 2.2 times more insulin into the medium than cells exposed to 16.7 mM glucose over the whole 48 h stimulation period. The insulin content of chronic high pyruvate-treated cells was reduced by 40%. However, the mitochondrial membrane was still hyperpolarized in response to 16.7 mM glucose and the plasma membrane potential increased to -61 ± 3.5 mV similarly to control cells.

Conclusion: After chronic high glucose exposure, the insulin secretory response of the beta cells is greatly perturbed. Several factors such as reduced insulin content, altered glucose metabolism and impaired mitochondrial function appear to be involved. However, despite of fuel-overload of mitochondria during cultivation of INS-832/13 cells with supraphysiological pyruvate concentrations, the beta cells remained excitable and maintained their secretory response to glucose. We suggest that extramitochondrial factors, due to perturbed glucose metabolism upstream of pyruvate, largely contribute to a perturbation of fuel-dependent regulation of insulin secretion.

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Phospholipid remodelling, augmented peroxidation of polyunsaturated fatty acids and activation of PPAR- δ mediate glucose-amplified insulin secretion

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Background and aims: Intermittent periods of hyperglycemia activate 'priming' or 'memory' pathways that amplify insulin release from β -cells. This phenomenon may trigger the compensatory and adaptive phases of β -cell function in the early stages of type 2 diabetes. Recently, a role for PPAR δ as a lipid sensor that regulates insulin secretion has been proposed. We addressed the hypothesis that glucose-induced release of polyunsaturated fatty acids (PUFAs) from membrane phospholipids and their subsequent peroxidation generate bioactive lipid mediators that amplify insulin secretion from β -cells in a PPAR δ -dependent manner.

Materials and methods: High glucose-dependent release of fatty acids from β -cell membrane phospholipids was determined by lipidomic analysis. HPLC was used to identify peroxidation products of PUFAs in β -cell cultures. Static and dynamic glucose-stimulated insulin secretion assays were performed on isolated rat pancreatic islets and INS-1E cells. The role of PPAR δ in this process was ascertained with pharmacological agents and by manipulating PPAR δ expression in β -cells, and with a PPAR-Response-Element (PPRE)-dependent transactivation of luciferase gene.

Results: High glucose incubations (24h) activated cPLA2 in INS-1E cells, which consequently released linoleic acid and arachidonic acid from membrane phospholipids. Glucose-derived reactive oxygen species enhanced the non-enzymatic peroxidation of these fatty acids and augmented the generation of the bioactive aldehyde 4-hydroxy-2E-nonenal (4-HNE). Similar to GW501516 (a selective PPAR δ agonist), 4-HNE increased insulin secretion from INS-1E cells and isolated rat islets; these effects were blocked by GSK0660 (a potent PPAR δ antagonist) or by silencing PPAR δ expression. High glucose, GW501516 or 4-HNE markedly induced luciferase activity in INS-1E cells expressing the PPAR δ -PPRE transactivation system.

Conclusion: This study suggests that β -cells respond to high glucose exposures by remodeling membrane phospholipids and releasing PUFAs. Enhanced peroxidation of both linoleic acid and arachidonic acid generates 4-HNE, an endogenous ligand for PPAR δ . The activation of this nuclear receptor then augments insulin secretion in a manner characteristic of the adaptation of β -cells to hyperglycemic episodes.

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Novel mechanistic link between focal adhesion remodelling and glucose-stimulated insulin secretion

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Background and aims: Actin cytoskeleton remodelling is well known to be positively involved in glucose-stimulated pancreatic beta cell insulin secretion. We have observed glucose-stimulated focal adhesion remodelling at the beta cell surface and have shown this to be crucial for glucose-stimulated insulin secretion (GSIS). However, the mechanistic link between such remodelling and the insulin secretory machinery remained unknown and was the major aim of this study.

Materials and methods: All experiments were performed on MIN6B1 cells posed on 804G matrix (having demonstrated previously that under these conditions MIN6B1 behave similarly to primary beta cells regarding focal adhesion remodelling in response to glucose). For immunoprecipitation and protein phosphorylation, MIN6B1 were incubated for 2h at 0 mM glucose followed by 20 mM glucose for 10 min. RNAi was used to knockdown paxillin expression: cells were transfected (Lipofectamine 2000) 3 days prior to testing GSIS (2 h pre-incubation at 0 mM glucose; 1 h 0 mM glucose (basal); 3–60 min 20 mM glucose (stimulated)).

Results: Total internal reflection fluorescence (TIRF) microscopy revealed the glucose-responsive co-localisation of Focal Adhesion Kinase (FAK) and paxillin with integrin β 1 at the basal cell surface after 10 min of stimulation. Blockade of the interaction between β 1 integrins and the ECM with an anti- β 1 integrin antibody (Ha2/5) inhibited glucose-induced phosphorylation of FAK (Tyr-397), paxillin (Tyr-118) and ERK1/2 (Thr-202/Tyr-204) by 50.7 ± 1.0 ($p < 0.001$), 56.5 ± 5.1 ($p < 0.01$), and $68.3 \pm 11.8\%$ ($p < 0.05$) respectively. A 78.6 ± 5.1 ($p < 0.001$), 86.7 ± 3.3 ($p < 0.0001$) and $42.1 \pm 12.6\%$ ($p < 0.05$) inhibition in glucose-induced phosphorylation of FAK, paxillin and ERK1/2 respectively, was observed when cells were treated with the actin filament stabilizing compound jasplakinolide (5 μ M). Furthermore, both pharmacological inhibition of FAK by compound Y15 (1 μ M) and RNAi-mediated knockdown of paxillin inhibited glucose-induced phosphorylation of ERK1/2 (Thr-202/Tyr-204) and its direct substrate synapsin 1 (Ser-62/Ser-67) coinciding with the inhibition of both early and late phases of GSIS. The t-SNARE SNAP-25 was found in newly formed focal protrusions in close proximity to FAK, paxillin at the extremity of actin fibres. GSIS is known to involve the disassociation SNAP-25 with actin and we postulated that FAK may be involved in this process. This was confirmed by FAK inhibition by compound Y15 that resulted in a $73.3 \pm 24.3\%$ ($p < 0.05$) increase in SNAP-25/actin association (assessed by co-immunoprecipitation) compared to the control glucose-stimulated condition.

Conclusion: We show here that glucose-induced phosphorylation and activation of FAK, paxillin and ERK1/2 in beta cells is mediated by β 1 integrins. Additionally, the data suggest FAK and paxillin to be upstream of glucose-induced actin cytoskeleton remodelling which leads to phosphorylation of ERK1/2 and its substrate synapsin 1, both known to be important in GSIS. FAK activation furthermore liberates SNAP-25 from its association with the actin cytoskeleton, allowing to participate in granule exocytosis. Taken together, these results indicate a novel mechanistic link between focal adhesion remodelling and the insulin secretory machinery in pancreatic beta cells.

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Exocytotic release of ATP triggers diacylglycerol spiking in insulin-secreting cells

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Background and aims: The lipid diacylglycerol (DAG) plays an important role in various signaling processes by membrane recruitment and activation of C1-domain-containing proteins, such as different isoforms of protein kinase C (PKC). DAG is generated in the β -cell plasma membrane in response to receptor and nutrient stimuli, and DAG-mediated activation of PKC amplifies insulin secretion. The aim of this study was to characterize the spatio-temporal pattern of DAG signals in β -cells and to clarify the mechanism underlying plasma membrane DAG generation by glucose and depolarizing stimuli.

Materials and methods: A biosensor based on the two adjacent DAG-binding C1 domains of rat protein kinase C γ tagged to green fluorescent protein

(C1aC1b-GFP) was used to monitor DAG in individual living MIN6 β -cell. The probe redistributes to the plasma membrane (PM) upon DAG formation, and this translocation was monitored on-line with confocal or evanescent wave microscopy. A proline in each of the C1 domains (positions 20 and 85) was replaced by glycine to yield a mutant sensor with markedly reduced affinity for DAG.

Results: Confocal imaging of MIN6 β -cells expressing C1aC1b-GFP showed diffuse cytoplasmic fluorescence under basal conditions. The probe redistributed to the plasma membrane in response to 1 μ M of the functional DAG mimetic phorbol myristate acetate. This translocation was detected as a marked increase of PM fluorescence with evanescent wave microscopy. A step increase of the extracellular glucose concentration from 3 to 11 mM triggered pronounced spikes of C1aC1b-GFP translocation with 3–16 s duration ($118 \pm 10\%$ fluorescence increase; $n=33$), occurring either irregularly or in bursts with a frequency of $0.25 \pm 0.1 \text{ min}^{-1}$ ($n=11$). No spiking was observed with the DAG-binding-deficient mutant sensor. Membrane depolarization with 30 mM K^+ induced an immediate increase of PM C1aC1b-GFP fluorescence followed by a decline and brief, high-amplitude spikes similar to those induced by glucose. Ca^{2+} omission prevented C1aC1b-GFP spiking, as did inhibition of exocytosis with 10 μ M adrenaline. Whereas 100 nM insulin had no effect, ATP induced detectable C1aC1b-GFP translocation at concentrations as low as 10 nM. Higher ATP concentrations (0.1–10 μ M) induced a dose-dependent initial rise of C1aC1b-GFP PM fluorescence ($89 \pm 11\%$ maximal fluorescence increase, $n=16$), followed by a decline to a stable plateau. Most of this effect was blocked by 10 μ M of the purinergic P2Y1 receptor antagonist MRS 2179, which also prevented C1aC1b-GFP fluorescence spiking induced by 30 mM K^+ or 11 mM glucose. Similarly, the DAG spiking was reversibly suppressed by exposure to a high ATP concentration (100 μ M) known to desensitize purinoceptors.

Conclusion: Glucose and depolarizing stimuli evoke pronounced DAG spiking in the β -cell plasma membrane. The effect is mediated by autocrine purinoceptor-phospholipase C activation by ATP co-released with insulin from the secretory vesicles. These complex DAG signals should have implications for kinetic control of exocytosis and other cellular functions.

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Role of glucose-6-phosphate in regulation of glucose-stimulated gene expression in the pancreatic beta cell line INS-1E

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Background and aims: In the liver, glucose (Glc) induces expression of glycolytic and lipogenic genes by activating the carbohydrate response element-binding protein (ChREBP). Further, ChREBP is dephosphorylated and activated by protein phosphatase 2A, which in turn is activated by xylulose-5-phosphate, an intermediate metabolite of the pentose phosphate pathway. In pancreatic islets, Glc induces metabolic gene expression in a manner similar to that observed in primary rat hepatocytes; however, pentose phosphate shuttle flux is very low in pancreatic beta cells. In adipocytes, glucose-6-phosphate (G6P) plays an important role in regulation of Glc-stimulated fatty acid synthase gene expression. In this study, we confirm the role of G6P in regulation of Glu-stimulated gene expression in the pancreatic beta cell line INS-1E.

Materials and methods: After a 24-h preincubation in a medium containing 2.5 mM Glc, INS-1E cells were further incubated for 2 h under three different conditions; 2.5 mM Glc, 25 mM Glc and 25 mM 2-deoxyglucose (2DG). 2DG is converted to 2-deoxyglucose-6-phosphate (2DG6P) but is not further metabolized by the glycolytic pathway. The cells were analysed using DNA microarray, TaqMan PCR, ChIP assay and reporter assay.

Results: Using DNA microarray, we observed that expression of 194 known genes changed significantly at a threshold of 2-fold on switching from 2.5 mM Glc to 25 mM Glc conditions. Of these, expression of 62 genes was induced at a threshold of 2-fold under 25 mM 2DG conditions, and the expression of 46 genes remained unchanged or repressed under this condition. Out of these 194 glucose-response genes, 9 genes were previously reported as ChREBP target genes, such as liver-type pyruvate kinase (Lpk) and thioredoxin-interacting protein (Txnip). In contrast, expression of 173 known genes changed at a threshold of 2-fold on switching from 2.5 mM Glc to 25 mM 2DG conditions. Of these, the expression of 25 genes remained unchanged or repressed under 25 mM 2DG conditions. Out of these 173 glucose-response genes, only 2 genes were previously reported as ChREBP target genes, such as Class E basic helix-loop-helix protein 40 (Bhlhe40) and thioredoxin-interacting protein (Txnip). 2DG6P concentration in the medium containing 25 mM 2DG was much higher than G6P concentration in the medium containing 25 mM Glc. The potency of 2DG in inducing Lpk and Txnip mRNA was weaker and less persistent than that of Glc; this result was consistent with that of DNA microarray. Moreover, the reporter assay, which involved the pGL3 promoter with $3 \times$ Lpk and $3 \times$ Txnip ChoRE, and the ChIP assay, which involved the anti-ChREBP antibody, revealed that 2DG does not increase ChREBP transactivity in INS-1E cells. Finally, transfection of siRNA against ChREBP partly inhibited Glc-stimulated, but not 2DG-stimulated, Lpk and Txnip expression.

Conclusion: Compared with Glc, 2DG weakly induces Lpk and Txnip expression without modulating ChREBP transactivity. The role of G6P in Glc regulation of ChREBP target genes appears insignificant.

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Regulation of core clock genes in human islets

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Background and aim: Recent genome-wide association studies have demonstrated that there is an association between circadian clock function (CRY2) and development of metabolic diseases, such as Type 2 Diabetes. Therefore, we investigated how expression of core CLOCK genes is regulated in human islets from donors suffering from Type 2 Diabetes.

Materials and methods: Islets from human donors were received from the Nordic Center of Islet Transplantation. We had access to islets from 9 patients with Type 2 Diabetes and 57 non-diabetic donors [66 patients; 35 males, 31 females; Age 56 ± 10.7 ; BMI (kg/m^2) 26 ± 3.8 ; HbA1c 5.9 ± 1]. We used the Affymetrix GeneChip® Human Gene 1.0 ST assay following the Affymetrix standard protocol; transcript levels of selected genes were also determined

by qRT-PCR, using Tacman® assays. For analysis of glucotoxic conditions, the glucose medium concentration was kept at either 3.3 or 16.7 mM for 48 h. Lipotoxic conditions were induced by keeping the islets in medium supplemented with 1 mM palmitate complexed to 1% bovine serum albumin for 48 h.

Results: Microarray analysis showed that the core clock genes *CLOCK*, *PER1-3* and *CRY1-2* are expressed in human islets. The mRNA levels of *PER2*, *PER3* and *CRY2* were lower in Type 2 Diabetes donors. To investigate the functional relevance of these *CLOCK* genes, we correlated their expression to insulin secretion (fold change) and HbA1c levels. mRNA levels of *PER2* ($\rho=0.510$; $p=0.001$), *PER3* ($\rho=0.445$; $p=0.005$) and *CRY2* ($\rho=0.881$; $p=0.004$) correlated positively with insulin secretion. Among them, only *PER3* correlated negatively with HbA1c levels ($\rho=-0.345$; $p=0.037$). Furthermore, in vitro induction of pathogenetic conditions (gluco-lipotoxicity), *PER1-3* mRNA levels were reduced in non-diabetic human islets compared to untreated ones ($p=0.003$).

Conclusion: Our data demonstrate that core molecular clock components are being regulated in human islets. In addition, these data suggest that impairment of the circadian clock may be part of the islet pathophysiology of Type 2 Diabetes in humans.

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The metabolite profile underlying insulin secretion: involvement of the pentose phosphate pathway in beta cell stimulus-secretion coupling

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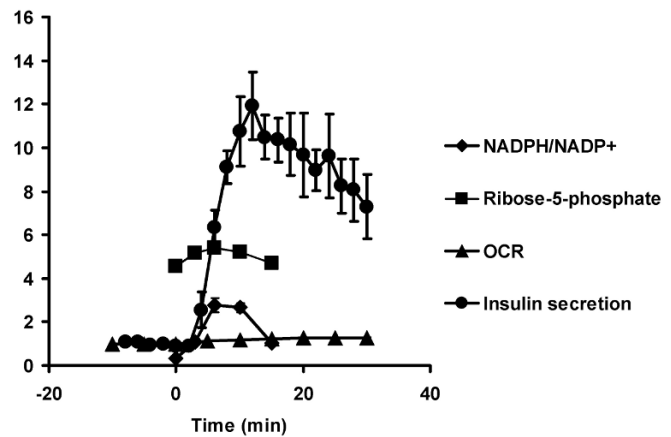
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Background and aims: Glucose stimulated insulin secretion is regulated by the triggering pathway (K_{ATP} -dependent) and the amplifying pathway (K_{ATP} -independent). Several metabolites, most of them emanating from mitochondrial metabolism, have been suggested to account for the latter mechanism. Conflicting results from these studies that mainly have focused on single metabolic pathways could be explained by the fact that only part(s) of cellular metabolism has been monitored. Here, we have attempted to characterize metabolic regulation provoked by glucose in a more global and unbiased fashion. Changes were assessed within 15 min after stimulation and correlated with levels of insulin secretion.

Materials and methods: INS-1 832/13 cells were pre-incubated at 2.8 mM glucose for two hours, followed by a 16.7 mM glucose stimulation. Metabolism was subsequently quenched at 0, 3, 6, 10, and 15 min after stimulation. Intracellular metabolites were extracted and analyzed by gas chromatography/time-of-flight mass spectrometry (GC/TOF-MS). In addition, oxygen consumption rate, NADPH/NADP⁺-ratio, static and dynamic insulin secretion was also assessed. Orthogonal projection to latent structures (OPLS) was used to correlate the metabolic profile with the level of insulin secretion.

Results: A clear correlation was observed between changes in the pattern of 195 detected metabolites and the level of insulin secretion. Analysis of this pattern revealed a rapid response in the major metabolic pathways of glucose, involving several previously suggested metabolic coupling factors. The complexity of metabolite changes observed disagreed with the concept of one single metabolite or pathway controlling insulin secretion. Instead, the complex alterations in metabolite levels suggest that a coupling signal should reflect large parts of the β -cell metabolic response. This was fulfilled by the NADPH/NADP⁺-ratio, which was transiently elevated at 6 min after glucose stimulation. Changes in this ratio paralleled the level of ribose 5-phosphate and coincided with rising insulin secretion. Inhibition of the pentose phosphate pathway abolished 15 min insulin secretion and reduced 60 min insulin secretion by 50%.

Conclusion: Our findings support involvement of NADPH and call for a reassessment of the role of the pentose phosphate pathway in β -cell stimulus-secretion coupling.



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Key role of AMPK in glucose-evoked Na,K-ATPase modulation

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Background and aims: Na,K-ATPase is an integral plasma membrane protein responsible for generating and maintaining transmembrane ionic gradients. In pancreatic β -cells, Na,K-ATPase is regulated by glucose and this regulation is impaired in glucose intolerant subjects, however, the underlying mechanism is still unclear. Since glucose has marked effects on intracellular ATP and AMP levels and AMP-activated protein kinase (AMPK), a key player in energy homeostasis providing exquisite sensitivity to small changes in AMP levels, the involvement of AMPK in the cascade of events regulating Na,K-ATPase in pancreatic β -cell was postulated. The aim of this work was to evaluate the putative role of AMPK in the glucose-evoked regulation of Na,K-ATPase activity in the pancreatic β -cell.

Materials and methods: Pancreatic β -cells from normal (control) or glucose-intolerant Wistar rats (GIR) were isolated and cultured (48h). After a pre-incubation (30min) with 2.1mM glucose (G2), cell batches were challenged with G2 or G8 (8.4mM glucose) for 20min, in the presence or absence of AMPK agonist (AICAR 1mM) and antagonist (compound C (CC), 10 μ M). Na,K-ATPase activity was assessed in intact cells by quantification of Pi, in the absence and presence of 1mM ouabain. Immunocytochemistry (ICC) of β -cells treated as previously described was performed using anti- α 1-Na,K-ATPase and anti-phospho(ser-23)- α 1-Na,K-ATPase antibodies. Western blots (WB) were performed in lysates of islets incubated in similar conditions plus AICAR or CC to evaluate α 1-Na,K-ATPase (ser-23) phosphorylation.

Results: In G2 the activity of Na,K-ATPase from control and GIR pancreatic β -cells was similar (0.184 ± 0.030 and 0.186 ± 0.020 μ molPi/min/mgProt, respectively). Challenging the GIR β -cells with G8 evoked a significantly lower inhibition (40%) of Na,K-ATPase activity compared to a 62% inhibition observed in control β -cells. In control β -cell, the addition of AICAR abolished glucose-induced Na,K-ATPase inhibition (0.166 ± 0.011 μ molPi/min/mg) whereas CC had no effect (0.063 ± 0.003 μ molPi/min/mg). In the contrary, in GIR β -cells CC significantly potentiated glucose-evoked inhibition of Na,K-ATPase to values similar to those observed in the controls (66%). WB analysis revealed that Na,K-ATPase- α 1 (ser-23) phosphorylation was increased by G8 (28 \pm 6% over basal) and abolished by AICAR. Additionally, CC induced an increase in phosphorylation equivalent to that observed in G8 (22 \pm 5% over basal). ICC showed an equivalent immunostaining intensity for α 1-Na,K-ATPase despite glucose concentration. However, for the phosphorylated (ser-23) α 1-Na,K-ATPase, a higher intensity was observed in cells exposed to G8 compared to G2.

Conclusions: The AMPK agonist AICAR counteracted the glucose inhibitory action on Na,K-ATPase from control β -cells whereas CC amplified the glucose-induced inhibition of Na,K-ATPase from GIR β -cells. These results suggest that AMPK plays a key role in the cascade of events regulating Na,K-ATPase and that the defect in GIR β -cells must be upstream of AMPK. AMPK inhibition by glucose metabolism and subsequent activation of PKC, phosphorylating Na,K-ATPase in ser23, may constitute steps of the mechanism underlying glucose-induced inhibition of Na,K-ATPase that might be uncoupled in GIR. Occurring prior to overt type 2 diabetes, this might be a feature of the disease development.

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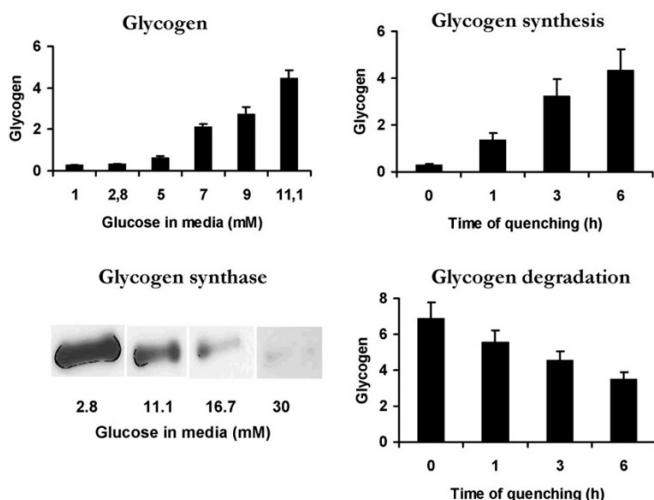
Glycogen metabolism in glucose-responsive and -unresponsive beta cells
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Background and aims: β -cell metabolism is suggested to be supply-driven to yield a dose-response of glucose-stimulated insulin secretion over a wide range of blood glucose concentrations. As a result, the rate of β -cell glycogen synthesis is assumed to be low. However, an increase in β -cell glycogen accumulation has been observed in type-2 diabetes (T2D). This has been proposed to reflect a pathogenetic role of glycogen metabolism in β -cells. This notwithstanding, no casual relation between glycogen synthesis and T2D has been established. We suggest that glycogen synthesis plays an important role in healthy β -cells and that this function is perturbed in T2D, resulting in excessive glycogen accumulation.

Materials and methods: INS-1 832/13 and INS-1 832/2 clonal β -cells were challenged at varying concentrations of glucose for 48 hours. Protein, mRNA, glycogen and metabolite analyses were performed to assess the regulation of glycogen metabolism and its impact on β -cell metabolism. Glycogen was quantified using a novel method based on gas chromatography/mass spectrometry.

Results: Our data show that glycogen synthase and phosphorylase are expressed at both the mRNA and protein levels in 832/13 β -cells. Furthermore, a dose-response of glycogen accumulation in response to increasing glucose concentrations was obtained. An abrupt increase in glucose concentration caused a fast and continuous accumulation of glycogen, reaching saturated levels already after 6 h. An abrupt drop in glucose concentration, on the other hand, resulted in a slow glycogen catabolism. Levels of glycogen synthase protein were found to decrease with increasing glycogen accumulation. The glucose unresponsive 832/2-cells did not produce glycogen, despite an increased stabilization of hypoxia induced factor 1 α (HIF-1 α). HIF-1 α responds to oxidative stress and has been suggested to be increasingly stabilized in T2D and to result in increased glycogen synthesis.

Conclusion: Our data suggest that glycogen synthesis in the β -cell functions to adjust β -cell metabolism to hypo- and hyperglycemia, the later presumably serving to hinder oxidative stress.



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Acute triggering of insulin release concomitant with a decrease in cytosolic free Ca^{2+} concentration by a combination of glucose and cAMP
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Background and aims: It is assumed that an elevation of cytosolic free Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) is mandatory for triggering insulin secretion in response to glucose, although glucose also exhibit KATP channel-independent amplifying effect on Ca^{2+} -triggered insulin release. Previously, we demonstrated that cAMP synergistically interacts with the KATP channel-

independent glucose action. In this study, we show that glucose can trigger prompt insulin secretion under conditions in which elevation of $[\text{Ca}^{2+}]_i$ is inhibited by the presence of diazoxide, a KATP-channel opener, and nifedipine, a calcium channel blocker.

Materials and methods: By using isolated rat pancreatic islets, we measured insulin release in static incubation and perfusion experiments. All experiments with some exceptions, insulin release was measured in the presence of 250 μM diazoxide and 10 μM nifedipine to inhibit Ca^{2+} -influx and to prevent an acute increase in $[\text{Ca}^{2+}]_i$. Forskolin was used to increase intracellular concentration of cAMP. Temporal profile of $[\text{Ca}^{2+}]_i$ was monitored with fura-2 loaded rat pancreatic beta cells in the presence of diazoxide and nifedipine.

Results: Under regular Ca^{2+} -containing (2.5 mM) conditions, 22 mM glucose stimulated insulin release by 10-fold for 30 minutes. Forskolin (10 μM) doubled insulin release in response to 22 mM glucose. Glucose-stimulated insulin release was abolished in the presence of both diazoxide and nifedipine as expected. In contrast, when forskolin was included during incubation experiments, 22 mM glucose induced a 5-fold increase in insulin release even in the presence of both diazoxide and nifedipine. This glucose effects is dependent on the presence of extracellular Ca^{2+} , because removal of Ca^{2+} from buffer deteriorated glucose action. These results suggest that glucose-induced Ca^{2+} -influx is not required for insulin release under the presence of diazoxide and nifedipine, but ambient Ca^{2+} is critical for it. Perfusion experiments revealed that 22 mM glucose increased the rate of insulin release within 2 minutes and peaked at 6 minutes after the stimulation followed by gradually increasing level of insulin secretion. The temporal profile of $[\text{Ca}^{2+}]_i$ changes are quite different from that of insulin release. Upon the stimulation with glucose, $[\text{Ca}^{2+}]_i$ sharply went down within 3 minutes. Thereafter, it went up almost lineally. The $[\text{Ca}^{2+}]_i$ level was kept at higher than basal level after 10 minutes of stimulation. Alpha-KIC (20 mM), a metabolizable nutrient, mimics glucose effect under the same condition. Metabolic inhibition of glucose by 2 mM NaN_3 strongly inhibited glucose effect. Cerulenin, an inhibitor of protein acylation, also inhibited glucose-induced insulin release in the presence of forskolin, nifedipine and diazoxide in a concentration-dependent manner.

Conclusion: Our results show that glucose and cAMP can trigger insulin release even when the KATP channel was forced to open and the voltage-dependent Ca^{2+} channel was closed. The effect was occurred concomitant decrease in $[\text{Ca}^{2+}]_i$. Glucose metabolism is required for the effects and protein acylation might be a responsible mechanism for triggering insulin release. A possible reason for extracellular Ca^{2+} dependency is that ambient Ca^{2+} concentration beneath the plasma membrane has permissive role for the triggering of insulin release.

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Glucose regulates free cytosolic Zn^{2+} concentrations by altering Slc39a and metallothionein gene expression in mouse pancreatic beta cells

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Background and aims: Zn^{2+} is an important cofactor for insulin biosynthesis and storage in pancreatic β -cells. Conversely, elevated intracellular Zn^{2+} levels are expected to be toxic. The high total concentration of Zn^{2+} in the pancreas suggests that its homeostasis may be tightly controlled by transporters and binding proteins (eg metallothioneins). There are two main families of zinc transporters: the ZnT family (coded by the Slc30a genes) diminish the concentration of the ion in the cytosol by transporting it either into the extracellular space or in intracellular organelles; the ZiP family members (coded by the Slc39a genes), on the other hand, act as importers localised either on the plasma membrane or on the membrane of intracellular compartments. Importantly, a polymorphism, rs13266634, in the human SLC30A8 gene encoding the secretory granule Zn^{2+} transporter ZnT8, is associated with altered type 2 diabetes risk. Despite recent finding, relatively little is known about how Zn^{2+} homeostasis is achieved in pancreatic β -cells and whether, and under what circumstances, changes in cytosolic Zn^{2+} ($[\text{Zn}^{2+}]_{\text{cyt}}$) may occur. Here, we explore the effect of glucose on $[\text{Zn}^{2+}]_{\text{cyt}}$ and the possible mechanisms through which the sugar is able to modify Zn^{2+} homeostasis.

Materials and methods: We used CD1 mouse pancreatic islets (for quantitative real time PCR and Western (immuno) blot analysis) or dissociated primary islet cells (for imaging). Real-time intracellular free Zn^{2+} measurements were achieved by adenovirus-mediated delivery into dissociated islet cells of a genetically engineered Förster resonance energy transfer (FRET)-based sensor, eCALWY-4, and epifluorescence imaging.

Results: We show here that elevated (16.7 vs 3 mM) glucose concentrations increase, in a time-dependent manner, free $[Zn^{2+}]_{cyt}$ in mouse pancreatic β -cells. These changes became highly significant (853 ± 96 pM vs 452 ± 42 pM, $p < 0.001$) after 24 h and were associated with increased expression of the ZiP family members Slc39a6, 7 and 8, and decreased expression of metallothionein-1 and -2 (Mt-1 and Mt-2). Arguing that the altered expression of these genes may be a cause of altered $[Zn^{2+}]_{cyt}$, an imposed elevation of extra (and intra-) cellular $[Zn^{2+}]$ failed to mimic the effects of glucose. By contrast, increases in intracellular cAMP, prompted by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine and the adenylate cyclase activator forskolin, partially mimicked the effects of glucose on metallothioneins, though not on ZiP expression. Furthermore, modulation of intracellular Ca^{2+} and insulin secretion by pharmacological agents (tolbutamide and diazoxide) suggested a possible role for the latter in the regulation of Slc39a6 and 7, but not Slc39a8 or metallothionein, expression by glucose.

Conclusion: We demonstrate that (1) glucose increases free $[Zn^{2+}]_{cyt}$ concentrations in mouse β -cells, presumably facilitating the processing and storage of insulin, and (b) these changes are achieved, at least in part, by modulation of the expression of metallothioneins and Zn^{2+} importers, albeit via distinct intracellular signalling mechanisms. Sustained hyperglycaemia, and the resulting increases in $[Zn^{2+}]_{cyt}$ may, however, contribute to β -cell dysfunction and death in type 2 diabetes, and these events may conceivably be modulated by ZnT8 genotype.

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Mathematical model of the mechanism by which alanine potentiates glucose-stimulated insulin secretion

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Background and aims: Pancreatic beta-cells maintain glucose homeostasis, secreting insulin according to blood nutrients' fluctuations. Amino acid alanine can acutely stimulate insulin secretion alone or synergistically enhance glucose-stimulated insulin secretion (GSIS) both in vivo and in vitro. A systems biology approach was employed to integrate biological experimental work with mathematical tools to gain novel insights about the role played by alanine in both stimulating on its own and enhancing GSIS.

Materials and methods: Experimental work was carried out on a functional clonal insulin-secreting cell line (BRIN-BD11) to validate the model and fine-tune the parameters. BRIN-BD11 cells were starved for 40 minutes at basal glucose level (1.1 mM) and then stimulated for 20 minutes with different concentrations of only glucose (1.1, 5, 16.7, 30 mM), only alanine (0.5, 1, 2, 5, 10 mM) and various combinations. Samples were assayed for consumption/production of key components of the mathematical model: glucose, alanine, lactate, glutamate, ATP and insulin. A mathematical model of GSIS in pancreatic beta-cells, based on mass action kinetics, which takes into account glycolysis, Krebs cycle, NADH shuttles and glutamate and alanine aminotransferase was built. The model's input is made up of glucose and/or alanine, while the output is ATP (as an increasing monotonic function between ATP production and insulin secretion was identified).

Results: Acute insulin secretion was concentration dependent with respect to glucose: increasing glucose concentration from 1.1 to 30 mM increased insulin secretion from 0.48 ± 0.09 to 0.83 ± 0.08 ng/mg protein/20min. Acute insulin secretion showed a robust dose-response curve with respect to alanine: increasing alanine concentration in the range 0.5–10 mM increased insulin release from 1.18 ± 0.11 to 2.43 ± 0.09 ng/mg protein/20min. The addition of 10 mM alanine significantly increased ($p < 0.001$) GSIS by 3.8–4.3 fold (2.08 ± 0.07 and 2.49 ± 0.05 ng/mg protein/20min at 1.1 mM and 30 mM of glucose, respectively). Both glucose consumption and lactate production showed a dose-dependent increase (from 0.13 to 3.02 and 0.057 to 0.16 μ mol/mg protein/20 min, respectively) which was significantly enhanced by addition of 10 mM alanine ($p < 0.001$). Alanine consumption showed a dose-dependent increase (from 0.08 to 1.22 μ mol/mg protein/20 min in the range 0.1–10 mM alanine), but was found unaffected by the presence of glucose intracellular glutamate concentration was 0.25 ± 0.04 , 0.32 ± 0.05 and 0.49 ± 0.1 1.22 μ mol/mg protein/20 min in the presence of 16.7 mM glucose, 10 mM alanine and their combination, respectively.

Conclusion: The model is able to describe both qualitatively and quantitatively the salient features of stimulus-secretion coupling in the beta cell. The model predicts high ATP production and thus strong insulin secretion if the

system is fuelled with either high glucose flux only or high alanine flux only, or both, confirmed by cell based experimental results. High ATP production levels are achieved at lower glucose concentrations when in combination with alanine. At high rates of alanine flux, a high steady state concentration of glutamate is achieved which predicts that alanine may enhance insulin release by increasing ATP levels and by generating glutamate, a putative messenger in insulin secretion.

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Glucose triggers cyclic Epac2 translocation and Rap activation at the beta cell plasma membrane

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Background and aims: Glucose stimulation of β -cells results in pulsatile release of insulin. This process is controlled by synchronized oscillations of the cytoplasmic Ca^{2+} and cAMP concentrations beneath the plasma membrane ($[Ca^{2+}]_{pm}$ and $[cAMP]_{pm}$). Important effects of cAMP are mediated by the guanine nucleotide exchange factor Epac2, which promotes insulin secretion through activation of the Rap family of small GTPases. The aim of the present study was to investigate spatio-temporal regulation of Epac2 and the effect of glucose stimulation on the activity of Rap GTPases.

Materials and methods: Evanescent wave fluorescence imaging was used to monitor plasma membrane association of fluorescent protein-tagged Epac2 in individual MIN6 β -cells. Mutants of Epac2 deficient in cAMP- and Ras-binding were generated by introducing G114E/G422D and K684E substitutions, respectively. Active (GTP-bound) Rap was visualized with a green fluorescent protein-tagged Rap-binding domain from RalGDS (GFP-RalGDS_{RBD}).

Results: cAMP-elevating agents and the Epac2-activating nucleotide analogue 007 caused translocation of GFP-Epac2 from the cytoplasm to the plasma membrane. Elevation of the glucose concentration from 3 to 11 mM often triggered cyclic translocation of Epac2, reflecting oscillations of $[cAMP]_{pm}$ and $[Ca^{2+}]_{pm}$. This translocation was suppressed after inhibition of adenylyl cyclases or removal of extracellular Ca^{2+} . Epac2 mutants deficient in cAMP- or Ras-binding failed to translocate in response to glucose stimulation or cAMP elevation, and expression of a dominant negative Ras mutant reduced translocation of wildtype Epac2. To investigate if Epac2 translocation was associated with activation of effector proteins at the plasma membrane, Rap activity was monitored with GFP-RalGDS_{RBD}. A step increase of the glucose concentration from 3 to 11 mM induced cyclic plasma membrane translocation of GFP-RalGDS_{RBD}. Activation of Epac with 007 or a combination of forskolin and IBMX induced stable translocation of the Rap reporter. Subsequent depolarization of the cells with 30 mM KCl, which promotes translocation of Epac2 to the plasma membrane, induced further GFP-RalGDS_{RBD} translocation.

Conclusion: Glucose stimulation of β -cells induces cAMP- and Ras-dependent Epac2 translocation to the plasma membrane, which in turn is associated with activation of the plasma membrane pool of Rap GTPases. Cyclic Epac2 and Rap signaling at the plasma membrane should contribute to the generation of pulsatile insulin secretion.

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Mitochondrial function parameters during metabolic amplification of insulin secretion

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Background and aims: The amplifying pathway of insulin secretion is known to involve the export of mitochondrial intermediates. However, it is neither known which metabolites are critical nor is there an undisputed target structure. Recently, we have observed that under an experimental condition which permits to compare the amplification by the nutrient secretagogues glucose and KIC (alpha-ketoisocaproic acid), the secretory response to glucose was entirely lacking while a virtually immediate response was elicited by KIC. This experimental situation should permit to gain further insight into nutrient signalling in the beta-cells.

Materials and methods: Primary mouse pancreatic islets were isolated by collagenase digestion and cultured for up to three days. When attached to a

glass cover slip they were inserted into a purpose-built perfusion chamber on the stage of an epifluorescence microscope. Successively, the fluorescence of NADH, FAD and tetramethylrhodamine ethyl ester (TMRE; as indicator of the mitochondrial membrane potential) were excited and registered by a photon-counting multiplier. ATP and ADP were determined by luciferase luminescence. Insulin secretion of batch-perfused islets was determined by ELISA.

Results: After perfusion for 1 h in the absence of glucose (to down-regulate the beta cell glucose memory) and the presence of 2.7 μmol glipizide (to close KATP channels), maximally effective concentrations of the following nutrients were added: glucose (30 mM), KIC (10 mM) or KIV (alpha-ketoisovalerate; 10 mM). While glucose had no insulinotropic effect under this condition KIC led to a 10-fold increase within 10 min, which slowly declined thereafter. KIV had a fast monophasic effect which returned to basal within 30 min. Despite the lack of insulinotropic effect, glucose still markedly increased the NAD(P)H autofluorescence (70% compared to prestimulatory values) and hyperpolarized the mitochondrial membrane potential (40% increase of TMRE fluorescence). Similar, but less extensive changes were produced by KIC (20%), while KIV was the least effective in this regard. The ATP- and ADP-contents after glucose or KIC incubation were not significantly different. There was however, a qualitative difference in the FAD autofluorescence, which was reduced by glucose (about 10%), increased by KIC and remained virtually unaffected by KIV.

Conclusion: Under a condition when the amplifying response to glucose is specifically abolished, the global mitochondrial function appears unimpaired. The qualitative difference in the FAD autofluorescence may indicate that the critical metabolite enabling amplification is derived from a pathway involving the FAD/FADH₂ redox couple.

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Ca²⁺-dependent regulation of Kv2.1 channels in pancreatic beta cells

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Background and aims: ATP-sensitive potassium (KATP) channel is known to determine the resting membrane potential that is approximately -70 mV at lower glucose level in pancreatic β -cells. In normal subjects, however, fasting plasma glucose level is maintained in the range of 5–6 mM. Under these conditions, ATP/ADP ratio is unchangeable because the glucose concentration is nearly constant. Thus, a fine-tuning of insulin secretion to maintain these plasma glucose levels in normal state should be explained with a factor other than KATP channel unless the KATP-channel is mechanistically involved in slow potential oscillations. We examined whether the voltage-dependent potassium channel (Kv channel) are regulated by cell metabolism including Ca²⁺ and energy metabolism.

Materials and methods: We measured the Kv-channel current in rat pancreatic β -cells or in HEK293 cells expressed with Kv2.1-channels by using whole-cell clamp mode. Insulin secretion was measured in islets in static incubation at 37°C for one hour. Electrophysiological experiments were performed at room temperature.

Results: Exposure of islets to TEA increased insulin secretion at 8.3 mM glucose (22.1 ± 1.1 ng/ml/10 islets vs 60.7 ± 5.6 ng/ml/10 islets, $P=0.0003$) but not at 2.8 mM glucose (10.1 ± 0.7 ng/ml/10 islets vs 9.3 ± 1.0 ng/ml/10 islets, $P=0.88$). When we compared the current-voltage relations of Kv channels recorded at 0 and 10 min after establishment of whole cell clamp mode using the pipette solution including 5 mM MgATP in the presence of 2.8 mM glucose in the external solution, Kv currents were increased at negative potentials between -40 mV and -10 mV ($P<0.01$). The same time-dependent increases in Kv-channel currents were observed upon exposure to 0 mM glucose, 1 μM FCCP, 0 mM ATP (in pipette) and 5 mM AMPPNP (in pipette). When we tested effects of presence of 5 U/ml alkaline phosphatase in the cell interior through the pipette, Kv-channel currents were increased at the same negative potential range ($P<0.01$) and activation and inactivation curves revealed a leftward shift. To test whether Kv2.1 channel is regulated by Ca²⁺, we applied high Ca²⁺ (1.5 μM , using two chelators two metal calculators) or low Ca²⁺ (16 nM) solution into the cytoplasm through the pipette in HEK293 cells expressed with Kv2.1 channels. Kv2.1-channel current was increased by Ca²⁺ increase at negative potentials ($P<0.02$) as observed in metabolically inhibited β -cells. This Ca²⁺-induced increase of Kv2.1-channel current was attenuated by a calcineurin inhibitor, 200 nM fenclerolate or 1 μM deltamethrin. In HEK293 cells without expression of Kv2.1 channels, Ca²⁺ increase did not change the background current. β -cell Kv channel currents were also increased by 1.5 μM Ca²⁺ in the pipette and the resting membrane potentials were hyperpolarized from -20.2 ± 1.4 mV at 16 nM Ca (n=8) to -33.2 ± 1.8 mV at 1.5 μM Ca²⁺ (n=9, $P<0.001$).

Conclusion: We conclude that the Kv2.1 channel in rat β -cells is mediated by glucose metabolism, phosphorylation/dephosphorylation (calcineurin) and cytoplasmic Ca²⁺. These Ca²⁺-dependent regulation of the channel may produce hyperpolarization of the membrane potential during glucose stimulation. This may consequently contribute to regulation of insulin secretion at intermediate glucose concentration in concert with KATP channel-based regulation of membrane potential.

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Low concentration of GLP-1 increases insulin secretion and calcium current by activating protein kinase C pathway in pancreatic beta cells

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Background and aims: GLP-1 is known to be the most potent promoter of insulin secretion in the body, and its concentration in human peripheral

blood is a few pM. Administration of DPP-4 inhibitors increases the peripheral blood concentration of GLP-1 by 2–3 fold, resulting in a significant potentiation of insulin release. On the other hand, almost all of *in vitro* experiments studying the effect of GLP-1 on pancreatic islet cells have routinely used much higher concentrations of GLP-1 (nM range) than physiological one. We have previously reported that a low concentration (1 pM) of GLP-1 stimulated insulin secretion through a cAMP-PKA independent pathway in MIN6 cells. In this study, we further assessed a precise action mechanism of physiologically low concentrations of GLP-1 on insulin secretion from primary cultured beta-cells.

Materials and methods: Mouse islets were isolated by collagenase digestion. Secreted insulin was quantified using the RIA kit. Dispersed islet cells were plated on plastic tissue culture dishes for electrophysiological recordings. The recordings were made using the perforated-patch whole-cell configuration. Membrane currents were recorded using an EPC-9 patch-clamp amplifier (HEKA). The cells were voltage-clamped at -70 mV , and the voltage-dependent calcium channels were activated by 20-ms depolarizations to 0 mV.

Results: GLP-1 stimulated insulin secretion in a dose dependent manner. In mouse beta-cells, 1 pM GLP-1 increased calcium current (I_{Ca}) by approximately 120% of the control (2.38 ± 0.61 vs 2.85 ± 0.74 pC, $N=5$, $p<0.05$). The increase in I_{Ca} was completely inhibited by addition of 100 nM exendin9-39, the GLP-1 receptor blocker. Insulin secretion induced by 10 nM GLP-1 was completely inhibited by 100 μM Rp-cAMPS (PKA inhibitor), while the effect of 1 pM GLP-1 was not significantly affected (16.3 ± 1.6 vs 23.0 ± 4.0 pg/islet/h, $N=7$). The stimulatory effect of 1 pM GLP-1 on I_{Ca} was still observed (116% of the control; 2.06 ± 0.20 vs 2.39 ± 0.19 pC, $N=5$, $p<0.05$) even in the presence of the PKA inhibitor Rp-cAMPS. The PKC inhibitor, 100 nM bisindolylmaleimide, completely blocked the effect of 1 pM GLP-1 on insulin secretion and I_{Ca}. Insulin secretion stimulated by GLP-1 was significantly inhibited by 2 μM isradipine, a L-type calcium channel blocker (49.6 ± 7.0 vs 20.2 ± 2.4 pg/islet/h, $N=7$). Surprisingly, GLP-1 further decreased calcium currents in the presence of isradipine. Neither 1 pM nor 10 nM stimulated insulin secretion from islets of GLP-1 receptor knockout mice.

Conclusion: The present results demonstrate that a low, but physiological, concentration of GLP-1 increases calcium currents and insulin secretion, and suggest that PKC is involved in its signal pathway, which is different from classical PKA dependent pathway. Further study should be required to identify the site of action of both higher and lower concentrations of GLP-1. Investigating the effects of physiological concentration of GLP-1 may contribute to bring forward the action mechanism of GLP-1 as an anti-diabetic agent.

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Fast and easy evaluation of beta cell function and activity

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Background and aims: Glucose-induced electrical activity of beta-cells in intact islets consists of membrane potential (V_m) oscillations, so-called slow waves. Bursts of Ca^{2+} action potentials trigger oscillations of $[\text{Ca}^{2+}]_c$ which in turn induce oscillating insulin secretion. The length of these oscillations is glucose dependent at concentrations between ~6–20 mM. Thus, the fraction of plateau phase (FOPP = percentage of time with burst activity) is an excellent marker of beta-cell activity and metabolic integrity. So far all tools to measure the FOPP require high technical skills, are invasive, and/or very time-consuming. Our aim was to determine the FOPP in islets of Langerhans with a non-invasive easy-to-use method.

Materials and methods: Changes of V_m of whole islets were recorded as field potentials using microelectrode arrays (MEA). For comparison V_m of beta-cells was recorded with the patch-clamp technique.

Results: Glucose-induced slow waves of mouse beta-cells exhibited very similar patterns of electrical activity regardless whether they are recorded by extra- or intracellular techniques. Extracellular recordings could be sufficiently resolved to clearly discriminate burst and interburst phases, enabling the precise determination and calculation of the FOPP. The concentration-FOPP curve was measured for glucose concentrations of 3 mM ($n=10$), 6 mM ($n=5$), 8 mM ($n=5$), 10 mM ($n=8$), 15 mM ($n=11$), and 30 mM ($n=5$). The data were fitted with the Hill equation giving a half-maximal glucose concentration of 12 ± 1 mM, similar to “classical” recordings of V_m , $[\text{Ca}^{2+}]_c$ or insulin secretion. Modulators of K_{ATP} channels (diazoxide ($n=8$) and tolbutamide ($n=8$)) changed the FOPP according to their well known action on the

channels. Moreover, TRAM34 (1 μM), an inhibitor of SK4 channels, increased the FOPP 1.25fold ($n=9$).

Conclusion: Here we present a new method using MEA technology as an easy-to-use tool to measure electrical activity from pancreatic beta-cells in intact islets of Langerhans. The simplicity of the method promises versatile applications from academic research to the development of medium-throughput systems for non-academic beta-cell research which may considerably profit from a routine use of this method. Importantly, the new technique may suit to test islets for metabolic integrity before transplantation.

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Hybrid bioelectronic sensor development for long-term functional screening on islets and insulin therapy improvement

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Background and aims: Sensor technology for insulin therapy has made considerable progress but hormonal regulation, detection of hypoglycaemia and real-time as well as closed-loop functioning are still open questions. Innovative sensors should also considerably advance long-term functional screening of healthy or diseased β -cells/islets. We report here the development of a hybrid biosensor based on the combination of electrophysiological recording of islet cells with multielectrode arrays (MEAs) and microelectronics devices.

Materials and methods: Clonal β -cells and mouse islets were cultured for 2–7 days on MEAs containing 60 extracellular microelectrodes (Multichannel Systems, MCS). Electrical signals were recorded with a MEA1060-Inv-BC-Standard amplifier (MCS) and off-line analyzed with MC_Rack software (MCS). An innovative low-power integrated circuit was also developed to optimize the real-time detection of spikes with low signal-to-noise ratio, the detection being based on wavelet transforms followed by adaptive thresholding.

Results: We succeeded for the first time in the culture and the long-term recording of electrical signals of both clonal and primary β -cells on MEAs. In clonal β -cells, spike frequencies increased in response to glucose in reversible and reproducible manner. In addition, glucose effects on spike frequencies were dose-dependent for concentrations mimicking hyper- and hypoglycaemia. The intestinal hypoglycaemic incretin hormone GLP-1 as well as the incretin-mimetic agent forskolin increased the firing rate of clonal β -cells. On the other hand, the hyperglycaemic hormone adrenalin, released during high-glucose-consuming situations, decreased electrical signals generated by primary β -cells and increased those from α -cells. The device is thus well-suited for simultaneous electrophysiological investigations on different islet cell types from the same biological sample. Functional monitoring over several days was feasible as we measured electrical responses of clonal β -cells to glucose on the same MEAs, 3 and 6 days after seeding. Glucose-evoked firing rates were reduced in β -cells exposed to glucotoxicity during these 3 days as compared to those exposed to normal glucose levels. In addition, we have shown the feasibility of acute pharmacotoxicological screenings on β and non β -cells with several compounds targeting islet ion channels such as nifedipine, tetraethyl ammonium, iberiotoxin, stomatocytin-1 and tetrodotoxin. The device was also used for electrophysiological phenotyping using islets from glucose-intolerant knock-out mice (adenylyl cyclase 8). Finally, algorithms were designed to improve the detection of very small amplitude spikes using adaptive thresholds after wavelet transform. A dedicated very large scale integrated circuit was manufactured, compatible with the MEA-amplifier and with adjustable approximation orders and thresholds. Real-time detection performances of the hybrid bioelectronic sensor were successfully tested online in *in vitro* experiments with clonal β -cells.

Conclusion: Our results demonstrate the feasibility of long-term functional screening on islet cells with MEAs, the improvement of real-time spike detection by online microelectronics, and the interest of hybrid biosensor developments for high-throughput screening and for the treatment of diabetes.

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Interplay between sub-plasma membrane oscillations of Ca^{2+} and ATP in mouse beta cells

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Background and aims: ATP plays an important role in β -cell stimulus-secretion coupling by linking glucose metabolism to closure of K_{ATP} channels, which results in membrane depolarization, Ca^{2+} influx and insulin secretion. Substantial evidence indicates that β -cell metabolism oscillates, and that such oscillations are affected by the cytoplasmic Ca^{2+} concentration. However, Ca^{2+} may exert both positive and negative effects on ATP levels and the aim of the present study was to clarify how the ATP dynamics is influenced by the cytoplasmic Ca^{2+} concentration beneath the plasma membrane $[\text{Ca}^{2+}]_{\text{pm}}$ in individual β -cells.

Materials and methods: Islets isolated from C57BL/6 mice were infected with adenovirus expressing the fluorescent ATP sensor perceval, which is based on circularly permuted Venus fluorescent protein fused to the ATP-binding bacterial protein GlnK1. $[\text{Ca}^{2+}]_{\text{pm}}$ was recorded with the fluorescent indicator Fura Red. Fluorescence was recorded from the sub-plasma membrane space with total internal reflection microscopy.

Results: Perceval fluorescence was stable in most islet cells exposed to 3 mM glucose. Elevation of the glucose concentration to 11 mM induced an immediate, pronounced ($144 \pm 5\%$) increase of fluorescence, typically with superimposed oscillations with a frequency of $0.28 \pm 0.02 \text{ min}^{-1}$ ($n=25$ cells). Hyperpolarization of the glucose-stimulated cells with the K_{ATP} channel opener diazoxide or inhibition of voltage-dependent Ca^{2+} channels with methoxyverapamil resulted in an additional marked increase of perceval fluorescence, sometimes with remaining small oscillations. Subsequent depolarization with 30 mM K^{+} instantly reduced perceval fluorescence with small oscillations occurring in about 50% of the cells. Simultaneous measurements of $[\text{Ca}^{2+}]_{\text{pm}}$ showed that the initial glucose-induced rise of perceval fluorescence preceded the increase of $[\text{Ca}^{2+}]_{\text{pm}}$. Subsequent oscillations were anti-synchronous, with each increase of $[\text{Ca}^{2+}]_{\text{pm}}$ coinciding with lowering of perceval fluorescence.

Conclusion: Glucose stimulation of mouse β -cells triggers oscillations of the ATP concentration in the sub-plasma membrane space. Ca^{2+} reduces the ATP levels, but metabolic oscillations can occur without $[\text{Ca}^{2+}]_{\text{pm}}$ oscillations. The dynamic interplay between ATP and $[\text{Ca}^{2+}]_{\text{pm}}$ in β -cells should be important for the generation of pulsatile insulin secretion.

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Actions of the BK channel blocker charybdotoxin on glucose-induced electrical activity in whole mouse islets

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Background and aims: Bursting electrical activity is a distinctive trait of β -cells in intact islets, related to oscillatory Ca^{2+} influx and pulsatile insulin release. The possible physiological role of the large-conductance Ca^{2+} -sensitive K^{+} (BK) channel as a 'bursting channel' or modulator of action potential (AP) firing has been challenged by the reiterated lack of effect of prototypical peptide inhibitors on the electrical activity of whole islets. We have now investigated the effects of charybdotoxin (ChTx, 50 nM) on β -cell spiking activity, recorded from rather superficial islet cell layers to enhance access of the peptide. The hydrophobic BK channel inhibitor penitrem A (PTA, 10–100 nM) was also used to probe pharmacologically cells located deeper within the islet.

Materials and methods: The membrane potential was recorded from micro-dissected mouse islets, using high-resistance microelectrodes.

Results: Both inhibitors greatly increased spike amplitude and reduced (i.e. made more negative) the burst plateau potential in 11 mM glucose, effects that peaked at 10–15 min. These actions probably reflect a reduction in the voltage threshold for AP firing. In addition, both ChTx and PTA accelerated spike repolarization, increased spike afterhyperpolarization and augmented the inter-spike interval, effects that probably reflect enhanced activation of delayed rectifier K^{+} (K_{v}) channels and/or K_{Ca} channel types distinct from BK channels. Similar effects were observed in presence of 11 mM glucose and tolbutamide (500 μM), a blocker of ATP-sensitive K^{+} channels which depo-

larizes β -cells and generates a pattern of continuous spiking activity. Both inhibitors slightly reduced burst duration in 11 mM glucose alone, although in some experiments the electrical activity became so disrupted that this parameter was difficult to quantify.

Conclusion: Superficial intracellular recording revealed potent actions of charybdotoxin on β -cell electrical activity. BK channels contribute to shaping action potential firing by controlling its amplitude and frequency, being essential to sustain burst plateau and regularity.

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 K_{ATP} channel and mitochondrial pH: estimation of mitochondrial matrix pH in cultured living beta cells using an improved ratiometric biosensor

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Background and aims: Mitochondrial pH and changes in pH are key determinants of mitochondrial energy metabolism, and metabolite transport is important for cell activation. However, methods to estimate mitochondrial pH in living cells are suboptimal and do not provide a direct quantitative measurement. Here we report a new ratiometric biosensor which we employed to calculate organelle pH in living cells using fluorescence. Because β cell mitochondrial function is vital for insulin secretion, we used the new system to measure mitochondrial pH in two β cell lines, INS-1 (rat) and MIN6 (mouse).

Materials and methods: The pH measurement probe MitpHGV thus comprised two probes, a pH-dependent mutant fluorescent Venus with a pKa ranging from 7.2 to 8.0, and a pH-independent fluorescent mutated GFPuv. A cytochrome-C subclass IV localization signal was fused with our pH-sensitive probe (MitpHGV; Fig.1A), then stably transfected into Chinese hamster ovary (CHO) cells or cultured β -cells, INS-1, and MIN6 cells, using Lipofectamine. Mitochondrial pH in these cells was calculated serially and quantitatively under conditions that stimulated the secretion of insulin. MitpHGV-INS1 and MitpHGV-MIN6 cells were incubated with 2 μM Fura-2AM and Ca signals were calculated as previously reported. MitpHGV-MIN6 cells were incubated for 45 min with 1 μM BCECF-AM with 0.0025% F127 added at room temperature and pH of the cytoplasm was monitored.

Results: Rotenone and carbonyl cyanide 3-chlorophenylhydrazone (CCCP) treatment quickly decreased mitochondrial pH in cultured β cells. After loading the cells with rotenone, however, the addition of CCCP increased mitochondrial pH. In cultured β cells, glucose and pyruvate also quickly decreased mitochondrial pH, and the pH recovered upon washout of these compounds. In contrast, treatment with arginine or tolbutamide without glucose induced a slow decrease in mitochondrial pH. Glucose (in a step change from 0 to 1.5 mM) also decreased mitochondrial pH in MIN6 cells, and further decreased with 25 mM glucose. On the other hand, treatment of MIN6 cells (in 0 glucose for 180min) with 1.5 mM glucose induced a Ca^{2+} response only in a subpopulation of MIN6 cells, while following 25mM glucose all MIN6cell showed a Ca^{2+} response. Furthermore mitochondrial pH decrease occurred following stimulation with 25mM Glucose even in the presence of diazoxide (100 μM).

Conclusion: We found that mitochondrial pH is decreased by insulinotropic stimulation in cultured β cells, and it occurs independent of functional K_{ATP} channels. Our new system for measuring intracellular pH using a targeted biosensor was sensitive across a physiologically useful range and will be a useful tool for evaluating mitochondrial function in insulin secreting cells.

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Involvement of K_{ATP} channels in the loss of beta cell function induced by human islet amyloid polypeptide

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Background and aims: Type 2 diabetes is characterised by islet dysfunction that lead to the impairment of insulin secretion. The presence of islet amyloid

deposition is a recognised hallmark in islets of type 2 diabetes patients. Amyloid deposits are formed by human islet amyloid polypeptide (hIAPP). There is now very strong evidence supporting the key role of amyloidogenesis in the progressive loss of pancreatic beta-cell mass and function. The main objective of this work is to investigate the mechanisms by which hIAPP overexpression could affect beta-cell function.

Materials and methods: We have used two models of long-term hIAPP overexpression. First, rat pancreatic beta-cell line, INS1E, was stably transfected with a hIAPP plasmid (hIAPP cells) and second, rat pancreatic islets were infected with a hIAPP lentivirus. To characterise the impact of hIAPP on beta-cell function, we measured insulin secretion in both models. Then, to determine by which mechanism hIAPP alters beta-cell secretory function, we studied in hIAPP cells, the intracellular Ca^{2+} mobilisation in response to glucose or sulphonylurea drug using Fura-2 labelling. By immunogold labelling, we studied the presence of intracellular hIAPP deposits (oligomers) and their interaction with ATP sensitive potassium channels (KATP) subunit, Kir6.2. Mitochondrial function was also evaluated by respirometry (OROBOROS system).

Results: In response to 16.7 mM glucose, insulin secretion was strongly decreased in hIAPP compared with control cells (3.5 ± 0.7 vs 15.6 ± 2.9 % insulin release expressed as a percentage of insulin content, $p < 0.05$). hIAPP-infected islets showed lower insulin secretion in response to glucose than control islets (1.7 ± 0.4 vs 2.5 ± 0.8 % insulin release expressed as a percentage of insulin content, $p < 0.05$). According to these results, the study of calcium signals demonstrated an absence of response to glucose in hIAPP cells. Whereas 76.61% of control cells (194/201) exhibited a normal response to 16.7mM glucose, none of the hIAPP cells tested were able to respond to glucose (0/192). Consistent with a defect in KATP channel function, hIAPP cells responded poorly to tolbutamide. Indeed, whereas 97.05% of control cells (132/136) showed a normal response to tolbutamide, only 9.6% of hIAPP cells (9/93) were able to respond. The immunogold labelling for oligomers was mostly detected in hIAPP cells. Double immunogold labelling for Kir6.2 and oligomers detected colocalisation of both proteins. In addition, the maximal respiratory capacity was increased in hIAPP cells compared to controls (79.62 ± 8.84 vs 46.56 ± 6.25 pmol/s/million of cells).

Conclusion: We suggest that hIAPP alters KATP channel activity leading to a defect in insulin secretion in response to glucose. The increase in mitochondrial activity may be a compensatory mechanism to counteract these defects. *Supported by: FIS (PI08/0088)*

PS 026 Islet mass and its regulation

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Enhanced functional capacity of human pseudoislets generated from the novel electrofusion-derived human insulin-secreting 1.1B4 cell line

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Background and aims: We have recently developed a novel human pancreatic beta cell line, designated 1.1B4, by electrofusion of freshly isolated of human pancreatic beta cells with immortal human PANC-1 epithelial cells. The current study describes, for the first time, the functional enhancement of these cells by configuration as human pseudoislets.

Materials and methods: Pseudoislet formation was achieved by seeding 1.1B4 cells at a density of 5×10^4 in ultra-low attachment, 6-well flat-bottomed plates with culture in RPMI-1640 for up to 7 days. Cellular insulin content and insulin secretory responses in acute 20 min incubations were determined by radioimmunoassay. Expression of gap junction and beta cell genes and proteins were measured by RT-PCR, Western blot and immunohistochemistry. Cellular proliferation and integrity were assessed by BrdU enzyme-linked immunosorbent assay and the colorimetric MTT assay.

Results: Pseudoislets formed readily over 3-7 days in culture attaining a static size of 100-200 μm , corresponding to approximately $6,000 \pm 417$ beta cells. Insulin content was comparable between monolayers and pseudoislets (approx. 3.2 ng/ 10^6 cells). Monolayers exhibited a stepwise 1.3-1.9 fold increase of insulin secretion at 0-16.7mM glucose which was enhanced 1.7-2.5 fold by pseudoislet formation ($p < 0.001$). Leucine, alanine, arginine and α -ketoisocaproic acid (all 20mM) stimulated insulin release from 1.1B4 monolayers by 2.4, 1.4, 2.2 and 2.1-fold ($p < 0.01$ to $p < 0.001$) respectively. However, cellular arrangement as pseudoislets greatly enhanced these responses, with insulin output attaining values 5.7 to 12.5-fold greater than monolayers ($p < 0.001$). Similarly, the gut hormones, GIP, GLP-1 and CCK-8 (all 10^{-9}M) significantly stimulated insulin release ($p < 0.01$ to $p < 0.001$) with 5.8-7.6 fold greater responses from pseudoislet configured 1.1B4 cells ($p < 0.01$ to $p < 0.001$). Evaluation of second messenger pathways showed that 30mM KCl, elevated extracellular Ca^{2+} (6.4mM), 25uM forskolin, 200uM IBMX or the K-ATP channel blocker tolbutamide (200uM) each stimulated insulin release ($p < 0.01$ to $p < 0.001$) from 1.1B4 cells with 2.8-5.8 fold greater responses from pseudoislets. Expression of E-cadherin, connexin 36 and connexin 43 were all enhanced in pseudoislets with significant differences ($p < 0.001$) evident from Western blot and immunohistochemistry analysis. Pseudoislets and monolayers exhibited similar levels of glucokinase gene expression, with significantly higher levels ($p < 0.01$) of the Glut1 glucose transporter detected in pseudoislets. Pseudoislets showed a 30% decrease in proliferation and a 21 % reduction in cell viability compared with monolayers ($p < 0.001$).

Conclusion: Novel electrofusion-derived 1.1B4 human beta cells readily formed pseudoislets in tissue culture. Enhanced beta cell to beta cell interactions mediated through the 3-dimensional cell formation and increases in gap junction communication, resulted in greatly improved secretory function. Such human pseudoislets may provide an attractive model for future islet research and represent an alternative limitless source of human-derived tissue for possible future cell replacement therapy of type 1 diabetes.

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The role of microRNAs in compensatory beta cell mass expansion

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Background and aims: Diabetes mellitus is a metabolic disease characterised by impaired glucose homeostasis resulting from defective function and/or loss of beta-cells. Pregnancy is associated with diminished insulin sensitivity of maternal tissues that is compensated by an expansion of the beta-cell mass and an increase in insulin release. A better understanding of this physiological process could help identifying new possible approaches for the treatment of diabetes. MicroRNAs are small non-coding RNAs acting as translational repressors. Some of these gene regulatory molecules are known to be involved in the control of beta-cell functions, including insulin secretion and apop-

tos. The aim of our study is to identify microRNAs that are differentially expressed in pancreatic islets in a context of insulin resistance and to assess their involvement in compensatory beta-cell expansion.

Materials and methods: We used expression profiling methods to compare the level of microRNAs in the islets of pregnant rats at d14 of gestation to non pregnant rats. Differential microRNA expression was verified by quantitative real-time PCR. The functional impact of selected microRNAs on insulin secretion, cell proliferation and survival was studied by modifying their expression in the insulin-secreting cell lines MIN6 and INS832/13 and in dissociated rat islet cells.

Results: Microarray and real time PCR analysis identified two down-regulated (miR-218 and -338-3p) and two up-regulated microRNAs (miR-144 and -451) in rat islets of pregnant rats. Interestingly, we found that miR-338-3p is also down-regulated in the islets of obese db/db mice and in high-fat fed mice, two other animal models characterized by compensatory beta-cell mass expansion. In vitro experiments with insulin-secreting cell lines and isolated rat islets revealed that miR-338-3p expression is diminished both in the presence of estradiol and upon exposure to the GLP-1 analogue exendin-4. Reduction of miR-338-3p expression in MIN6 and INS832/13 cells using antisense molecules resulted in an increase in proliferation, as evidenced by a rise in Ki67 and BrdU staining, without any impairment in glucose-induced insulin secretion. Moreover, anti-miR-338-3p treatment protected MIN6, INS832/13 cells and dissociated rat islet cells from apoptosis induced by prolonged exposure to pro-inflammatory cytokines.

Conclusion: We have identified changes in microRNA expression in pancreatic islets of pregnant rats. Our data suggest that miR-338-3p might be implicated in beta-cell compensation during pregnancy and in the setting of insulin resistance in different animal models. The identification of miR-338-3p downstream signalling pathways could provide important information for the design of new microRNA-based therapeutic strategies to expand the functional beta-cell mass.

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Relationships between pancreatic islet features and clinical characteristics

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Background and aims: To shed light on the relationships between pancreatic islets and clinical characteristics, we studied properties of islets from multiorgan donors, to be correlated with a few anthropometric and metabolic features

Materials and methods: Histological samples and/or isolated islets were obtained from 83 subjects; 56 non-diabetic individuals (Ctrl) [age: 61±16 yrs; 32 male (M) and 24 female (F); BMI: 26.2±3.8 kg/m² (n=49); mean blood glucose (BG) during ICU stay: 140±26 mg/dl (n=42)], and 27 type 2 diabetes mellitus (T2DM) patients [age: 67±8 years (p=0.01 vs Ctrl); 15 M e 12 F; BMI: 27.5±3.7 kg/m² (n=26) (p=0.1); BG: 219±50 mg/dl (n=21) (p<0.01)]. Morphometric analysis was performed by an Olympus microscope equipped with a specific software. Islets were prepared by enzymatic digestion and density gradient purification, and insulin secretion was assessed after stimulation with glucose, glibenclamide and arginine. An ELISA assay was used to evaluate cell death.

Results: Islet insulin-positive area was lower in T2DM subjects than in Ctrl (56.0±17.4% vs 71.9±9.5%, p<0.01), as it was insulin release in response to glucose (stimulation index, SI: 1.4±0.4 vs 2.8±1.6, p<0.01), glibenclamide (SI: 1.4±0.4 vs 2.7±1.5, p<0.01) and arginine (1.5±0.6 vs 2.5±1.1, p<0.01). The apoptotic rate was higher (0.88±0.72 vs 0.42±0.47 arbitrary units, p<0.01) in T2DM islets. Islet insulin area was positively correlated with basal (r=0.273, p<0.05) and arginine stimulated (r=0.320, p<0.01) insulin secretion from isolated islets, and was also inversely correlated with patients' BG levels (r=0.379, p<0.01). In addition, glucose and glibenclamide stimulated insulin release was inversely related to glycemic control (r=0.402, p<0.01). Finally, the higher the apoptotic rate, the lower the insulin area (r=0.248, p<0.05).

Conclusion: Correlations were found between pancreatic islet properties and donors' clinical characteristics, with beta cell amount seemingly representing an important variable for islet function and glucose control. These results confirm that the amount of beta cells is reduced in T2DM islets, probably due to increased apoptosis; more importantly, our data suggest that beta cell amount and glucose or glibenclamide stimulated insulin release are not related each other, although both contribute to control blood glucose levels.

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The ultrastructure of pancreatic beta cells in human type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) results from a combination of genetic and acquired factors which reduce insulin sensitivity and cause pancreatic beta cell damage. To date, very limited information is available as for the ultrastructure of beta cells in human T2D. In this study we performed morphological and morphometric electron microscopy evaluation of pancreatic islet cells in T2D and non-diabetic (ND) individuals.

Materials and methods: Pancreatic samples were obtained from the glands of 8 T2D and 5 ND organ donors, with similar clinical features, and processed by standard electron microscopy techniques. Morphometric analyses were performed on micrographs obtained at 10,000x and assessed by overlay with a 11x11 cm graticule composed of 169 points. Volume density was calculated by the formula: VD = Pi/Pt, where Pi is the number of points within the subcellular component and Pt is the total number of points, and expressed as ml/100ml of tissue (ml%). The total number of islet cells examined was 1405 in T2D and 2446 in ND.

Results: A lower amount of beta cells was found in T2D than in ND islets (62±2 vs. 71±3%, p<0.01), whereas no difference was observed for alpha (21±2 vs. 20±3%) and delta (5±1 vs. 4±1%) cells. More beta cells of T2D showed signs of apoptosis (7±1.6 vs. 2±1.3%, p<0.01). Insulin granules were less represented in T2D (3.8±0.4 vs. 5.9±0.6 ml%, p<0.01), and the amount of docked granules, although similar in diabetic (0.34±0.07 ml%) and non-diabetic (0.36±0.05 ml%) beta cells, showed a lower proportion of mature granules in the former (0.21±0.05 vs. 0.30±0.03 ml%, p<0.01). Volume density of the endoplasmic reticulum was increased in T2D samples (1.7±0.08 vs. 0.9±0.06 ml%, p<0.01), and mitochondria number (1.7±0.1 vs. 1.3±0.1 per 10 μm²) and volume (5.3±0.8 vs. 3.8±0.4 ml%) were also higher (both p<0.01) in diabetic than in non-diabetic beta cells.

Conclusion: These results show that in human T2D, beta cells have several ultrastructural alterations, as cause or consequence of functional and survival defects; targeting the deranged organelles might improve the outcome of therapeutic interventions.

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Direct evidence of adaptive changes of human islets to an obese murine environment

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Background and aims: Islet adaptation to obesity in rodents is well established yet no direct evidence exists proving human islet mass adapts to obesity. Indeed most obese people with insulin resistance do not develop diabetes because islets compensate by increasing insulin secretion to maintain normal blood sugar levels. Transplantation of human islets to immune-compromised mice is a validated technique to study human beta cells in an in vivo environment. The aim of this study is to show the adaptive changes of human islets to an obese murine environment.

Materials and methods: Twenty nine adult male C57Bl6 RAG 2 immunodeficient nondiabetic mice were transplanted under the kidney capsule with a suboptimal number of human islets (300 islet equivalents -IEQ) from four organ donors. Islets from one donor were transplanted into at least nine mice per condition. Animals were fed 12 weeks with Control or HFD (Research Diets USA). All mice were followed up for weight, 6 hour fasting glycemia and human c-peptide over 12 weeks. After the sacrifice, both endogenous murine pancreas and human grafts were fixed and paraffin embedded for morphometric analysis (endocrine mass, distribution of a,b cells, proliferation) or snap frozen for RNA extraction and gene expression analysis. Oral glucose tolerance tests (2g/kg body weight) were performed at 6 and 12 weeks. **Results:** HFD mice had increased body weights after 4-12 weeks versus controls (p<0.005), and higher serum triglycerides at 12 weeks. Fasting glycemia was higher in HFD mice from 6 weeks on (vs controls 6wk, 12wk, p<0.05). Human c-peptide levels (12wks: controls 1.606±0.54 vs HFD 3.69±0.69 ng/ml cpeptide p<0.005) or human cpeptide/ glycemia (12wks: controls 1.97±0.55 vs HFD 3.87±0.95 hu cpeptide/glycemia, p=NS) was consistently higher in HFD mice. Morphometric analysis of endogenous mouse pancreas re-

vealed doubling of islet mass in HFD mice vs controls. Planimetry confirmed the near doubling of human endocrine graft volume ($0.05\text{mm}^3 \pm 0.009$ vs $0.113\text{mm}^3 \pm 0.02$) and the increase of beta cells (%Beta cell surface/total endocrine surface: control $45.31\% \pm 2.004$ vs HFD $53.52\% \pm 0.1861$; $p=0.01$). Insulin gene expression was increased in grafts transplanted in mice on HFD. Total proliferation (BrdU positive/ total graft surface) was 4 times greater in HFD grafts (12wks) but endocrine and beta cell proliferation were not significantly different.

Conclusion: We exploited an immunodeficient mouse strain sensitive to hifad diet- induced obesity and show for the first time direct evidence that human islets adapt both endocrine and beta cell mass, function, and gene expression to obesity in vivo. This novel model allows for the first time a longitudinal study of human islet compensation to an obese murine environment and may be instrumental in deciphering pathways leading towards human islet expansion.

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Developmental programming of pancreatic tissue by maternal high fat feeding

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Background and aims: Overnutrition of pregnant rodents affects the development of the pancreas and susceptibility to diabetes in their offspring. We measured β - and α -cell mass in islets of Langerhans and pancreatic insulin content to obtain a deeper understanding of how pancreatic development is altered in offspring of over-nourished mice.

Materials and methods: Female C57BL/6 mice were fed either standard laboratory chow (C; fat 10% by energy) or an isocaloric high fat diet (HF; fat 42%) or for 8 weeks prior to mating. Offspring (referred to as C or HF according to their mother's diet) were all weaned onto chow. At 6 weeks of age the pancreata were harvested from male and female offspring from each maternal dietary group and processed for immunohistochemical analysis and total pancreatic insulin content. Immunohistochemistry was performed using the Aperio Genie™ for insulin and glucagon. Total pancreatic insulin was measured by radioimmunoassay. Data are expressed as mean \pm SEM.

Results: At conception the dams fed the high fat diet were heavier than the chow-fed dams (body weight: HF 31.4 ± 0.6 ; C 25.1 ± 0.4 g; $p<0.0001$) and fatter as measured by DEXA analysis (fat mass: HF 7.8 ± 0.4 ; C 2.5 ± 0.1 g; $p<0.0001$). At 6 weeks of age the body weights of male offspring from high fat-fed mothers were no different from those of chow-fed mothers (HF 25.6 ± 0.6 ; C 25.6 ± 0.3 g) but female HF offspring were surprisingly lighter than C offspring (HF 16.8 ± 0.5 ; C 18.8 ± 0.1 g; $p<0.0017$). Pancreatic weight expressed relative to body weight was higher ($p<0.05$) in HF offspring (HF female 0.95 ± 0.03 ; C female 0.62 ± 0.07 ; HF male 0.83 ± 0.14 ; C male 0.58 ± 0.02). In both male and female HF offspring, the number of islets, but not the average islet size, was increased (HF female 85.6 ± 10.1 ; C female 40.3 ± 8.7 ; HF 70.7 ± 6.3 , C 55.5 ± 9.3). HF offspring had an increased β -cell mass (HF female 0.38 ± 0.05 ; C female 0.27 ± 0.06 ; HF male 0.49 ± 0.07 ; C male 0.35 ± 0.08 mg) and an increased insulin area (HF female 0.32 ± 0.05 , C female 0.14 ± 0.03 ; HF male 0.32 ± 0.04 , C male 0.26 ± 0.06 mm²). α -cell mass was higher ($p<0.05$) in HF than in C female offspring (HF female 0.55 ± 0.12 , C female 0.19 ± 0.05 mg) with a larger glucagon area (HF female 0.32 ± 0.07 , C female 0.16 ± 0.04 mm²). Total pancreatic insulin was higher in HF than C offspring (HF female 9.05 ± 1.53 , C female 7.24 ± 0.37 ; HF male 10.5 ± 1.16 , C male 8.92 ± 0.66 microgram). By 27 weeks of age, despite no difference in pancreas weight, total pancreatic insulin content was increased ($p<0.05$) in HF female offspring when expressed relative to pancreas weight (HF female 119.35 ± 9.36 , C female 97.53 ± 5.11 microgram/g) or relative to body weight (HF female 0.8 ± 0.06 , C female 0.57 ± 0.06 microgram/g).

Conclusion: Maternal overnutrition remodels the endocrine pancreas, causing increased, islet number and α - and β -cell mass.

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Effect of intrauterine undernutrition during late gestation on pancreatic beta cell mass

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Background and aims: Impairment of insulin secretion in type 2 diabetes is accompanied by a decrease in the number of pancreatic β cells. A recent study reported that children with low birth weight are likely to have high risk of developing obesity, diabetes, hyperlipidemia, and hypertension in the future. Further, postnatal body weight gain, known as "catch-up growth," occurs in mammals (including humans) with low birth weight. Studies have shown that low-birth-weight rats whose mothers were maintained under poor nutritional conditions during late gestation have lesser number of pancreatic β cells than their normal counterparts. However, the longitudinal effect of undernutrition during the prenatal period on pancreatic β cell mass has not yet been elucidated. In this study, we developed a mouse model with low birth weight induced by starvation of pregnant mice.

Materials and methods: Pregnant C57BL/6J mice were subjected to 70% dietary restriction from late pregnancy (10.5 days postcoitum [dpc]) until delivery. The offspring were divided into 2 groups (food restriction group [RG] and control group [CG]) and were fostered to female ICR mice. At 4 weeks of age, both groups were provided high-fat diets. We examined the blood glucose and serum insulin levels and the pancreatic β cell mass to determine the effect of undernutrition on pancreatic β cells of the mice.

Results: The birth weight of RG offspring was significantly lower than that of CG offspring. Subsequently, RG offspring gained considerable body weight, and their body weight became equivalent to that of CG offspring about 7 days after birth. Pancreatic β cell mass in RG offspring at birth was about 30% less than that in CG offspring. At 8 weeks of age, a high-fat diet increased the pancreatic β cell mass in RG offspring compared to that in CG offspring, but the difference was not statistically significant. However, at 20 weeks of age, the β cell mass in RG offspring decreased and again became comparable to that in CG offspring. Blood glucose levels in RG offspring were higher than those of CG offspring at ≥ 8 weeks of age. However, blood glucose levels between 18-week-old RG and CG offspring did not differ significantly. Serum insulin levels in RG offspring were higher than those in CG offspring up to 20 weeks of age but tended to be lower at ≥ 24 weeks of age. The higher blood glucose and serum insulin levels and increased pancreatic β cell mass in RG offspring during the initial weeks after birth suggested that the offspring had impaired glucose tolerance.

Conclusion: These findings indicate that the growth of pancreatic β cells in mice with fetal undernutrition is insufficient, and pancreatic β cell failure can easily develop as a consequence of insulin resistance in these mice.

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Alterations in microRNA expression are potentially involved in the decline of function and mass of pancreatic beta cells in type 2 diabetes

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Background and aims: The mechanisms underlying the development of type 2 diabetes are diverse, all leading to the decline of function and/or mass of pancreatic beta-cells. MicroRNAs, a recently discovered class of non-coding RNAs, are potent regulators of key cellular processes, such as proliferation, differentiation and apoptosis. They are able to regulate gene expression by inhibiting the translation of target mRNAs through sequence-specific binding to the 3' untranslated region (3'UTR). In this study we evaluated the possible contribution of microRNAs to the decline of function and mass of pancreatic beta-cells in db/db mice, a well known type 2 diabetes model.

Materials and methods: Microarray analysis was employed to profile the expression of all known microRNAs in pancreatic islets of wild type and diabetic db/db mice. Two microRNAs showing a strong upregulation in the islets of diabetic mice were overexpressed in the mouse insulin-secreting beta-cell line MIN6B1 to test for their impact on insulin secretion and cell survival.

Bioinformatic tools were used to search for putative targets of these microRNAs and computational predictions were validated by western blotting upon overexpression of the microRNAs.

Results: We found that about 60 microRNAs display altered expression in the islets of diabetic db/db mice. In this study, we focused on miR-199a-5p and miR-199a-3p that are increased 12- and 9-fold, respectively, in the islets of diabetic db/db mice. Overexpression of miR-199a-5p in the beta-cell line MIN6B1 led to defective glucose-induced insulin secretion, while a rise in miR-199a-3p caused an increase in apoptosis. Western blot analysis revealed that overexpression of miR-199a-5p results in the upregulation of granuphilin a potent inhibitor of insulin secretion, and of Sirt 1 an NAD-dependent protein deacetylase that modulates cellular response to stress and altered metabolic flux. On the other side, overexpression of miR-199a-3p reduced the level of mTOR, a serine/threonine kinase playing a key role in beta-cell survival.

Conclusion: Our data suggest that miR-199a-5p and miR-199a-3p, two microRNAs issued from the same precursor, may contribute to beta-cell failure during instauration of type 2 diabetes: with miR-199a-5p causing defective insulin secretion, and miR-199a-3p having a negative effect on pancreatic beta-cell survival.

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PS 027 Beta cell death

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Activation of free fatty acid receptor FFA1/GPR40 by small agonists exerts beneficial effects on beta cell function and survival

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Background and aims: The insulinotropic effects of long chain free fatty acids (FFAs) including palmitate and oleate depend on the activation of free fatty acid receptor 1 (FFA1/GPR40) whereas adverse effects of FFAs rather depend on cellular accumulation and action of FFA metabolites. The G-protein coupled receptor FFA1/GPR40 couples to G_q and activates PLC and Ca^{2+} release from internal Ca^{2+} -stores. This study examined the effects of a small receptor agonist of FFA1 on insulin secretion and beta-cell survival and analysed the underlying mechanisms.

Materials and methods: The compound TUG-469 which contains a para-substituted dihydrocinnamic acid moiety and binds specifically to FFA1 was chosen to activate FFA1 in insulin secreting cells. Insulin secretion in response to TUG-469 was tested in static incubations of INS-1E cells, of isolated human islets and of WT and FFA1 knockout mouse islets. The release of Ca^{2+} from intracellular IP_3 -sensitive stores was inhibited by xestospongine, from ryanodine-sensitive stores by ryanodine. $[Ca^{2+}]_i$ was measured with the fluorescence dye fura-2. Apoptotic cell death was assessed by TUNEL and cleaved caspase-3 staining.

Results: In INS-1E cell, the FFA1 agonist TUG-469 (10 μ M) enhanced glucose (12 mM)-stimulated but not basal (2.8 mM glucose) insulin secretion. In aged, glucose-insensitive INS-1E cells TUG-469 still significantly augmented insulin release at high but not at low glucose. In parallel, TUG-469 increased $[Ca^{2+}]_i$ only at 12 mM but not at 2.8 mM glucose. Surprisingly, stimulation of secretion by TUG-469 was neither inhibited by ryanodine (1 and 100 μ M) nor by xestospongine (1 and 10 μ M). When INS-1E cells were cultured for prolonged time with TUG-469 (10 μ M for 2 d) no increase in apoptotic cell death was measured by TUNEL or cleaved caspase-3 staining. In contrast, palmitate significantly augmented apoptotic cell death of INS-1E cells and mouse islet cells. In islet cells from FFA1 KO mice palmitate induced apoptosis already at lower concentrations than in WT islet cells suggesting that FFA1 activation does not augment but even protects from apoptosis. In isolated human islets which express FFA1 TUG-469 (10 μ M) enhanced insulin secretion by 60 % and was, thus, as potent as palmitate.

Conclusion: These results suggest that the specific FFA1 agonist TUG-469 represents an attractive therapeutic tool to augment insulin secretion in a glucose-dependent manner.

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Mechanisms of autophagy activation in pancreatic beta cells

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Background and aims: In type 2 diabetes (T2D) a progressive decrease of beta-cell function and mass represent a common feature of the disease development. Beta cells, because of their continuous and sustained secretory activity, are constantly exposed to various kinds of stress from misfolded proteins, endoplasmic reticulum (ER) overload and mitochondrial damage. We have recently observed an autophagic vacuole accumulation in beta cells of T2D patients, associated with irreversible cell damage. This suggests that autophagy, a self-digesting mechanism normally responsible for removal of altered organelles and proteins, when exuberant or dysfunctional, might trigger non-apoptotic beta-cell death in diabetic patients. The aim of this study was to investigate the *in vitro* effects of increased levels of glucose and free fatty acids on autophagy activation in pancreatic beta cells.

Materials and methods: A well-differentiated beta-cell line (INS-1E) and isolated rat and human pancreatic islets were incubated for various time pe-

riods (from 0 to 24 h) at different concentrations of glucose (5, 11, 16.7 and 25 mM) and/or palmitic acid (0.1, 0.5 and 1.0 mM). Then, cell survival was evaluated and autophagy activation was explored by a) monodansyl cadaverine (MDC) and cathepsin D fluorescence; b) activation of LC3 protein; c) ultrastructural observation and morphometric analysis of autophagic vacuoles.

Results: In INS-1E cells and rat and human islets, autophagy was markedly activated in beta cells upon exposure to 0.5 and 1.0 mM palmitate (PA), but was not further enhanced by the concomitant presence of high glucose. High glucose alone was ineffective. In particular, the proportion of cells containing MDC-stained dots and the expression of the activated LC3-II band in Western blots considerably increased after 6 h of palmitate incubation. Moreover, LC3-II immunofluorescence co-localized with that of cathepsin D, a lysosomal marker, indicating that the autophagic flux was not impaired in PA-treated cells. Most of these effects were maintained up to 18–24 h incubation and were associated with a significant decline of cell survival, which was correlated with both palmitate concentration and incubation time. Ultrastructural analysis showed the presence of abundant autophagic vacuoles in PA-exposed beta cells, associated with a diffuse and remarkable swelling of the endoplasmic reticulum.

Conclusion: Our results clearly indicate that among the metabolic alterations occurring in T2D, high free fatty acids levels might play a major role in the activation of autophagy in beta cells, possibly leading to irreversible cell damage, through a mechanism that might involve the induction of ER stress.

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Apoptotic beta cell death independent of activation of transcription factor FOXO1: effects of glucocorticoids and the role of PKB/AKT isoforms

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Background and aims: Insulin receptor signalling pathways play an important role in maintenance of beta-cell function. The protective effects include the PKB/AKT-dependent phosphorylation of the proapoptotic transcription factor FOXO1. The phosphorylated form of FOXO1 is inactive due to its cytosolic retention. Our previous results suggest that the synthetic glucocorticoid dexamethasone (dexa) increased apoptotic beta-cell death, reduced PKB phosphorylation and increased the protein amount of FOXO1 which, unexpectedly, remained phosphorylated and cytosolic. We now performed experiments to determine the isoforms of PKB/AKT which phosphorylate FOXO1 and examined the role of FOXO1 during glucocorticoid-dependent apoptosis of insulin secreting cells.

Materials and methods: Expression levels of FOXO1 and of AKT1, AKT2 and AKT3 were analyzed by RT-PCR in insulin secreting INS-1E cells under control culture conditions and after treatment with dexa (100 nM) in the absence and presence of the glucocorticoid receptor (GR) inhibitor RU486 (1 µM). Protein levels and phosphorylations were examined by Western blotting. Knockdown of specific PKB/AKT isoforms was achieved by transfection with specific siRNA. Subcellular distribution of FOXO1 was examined after immunohistochemical staining using confocal microscopy. Apoptotic cell death was quantified by TUNEL and cleaved caspase-3 staining.

Results: INS-1E cells contain mRNA of PKB/AKT isoforms with the following order of expression levels: AKT1 (17.4 copies/GAPDHx1000) > AKT3 (3.8 copies/GAPDHx1000) > AKT2 (2.1 copies/GAPDHx1000). Dexa treatment did not change the expression levels of AKT isoforms but increased GR-dependent the expression of FOXO1 2.5-fold. Surprisingly, knockdown of AKT1 and AKT2 expression, either individually or in combination, neither affected phosphorylation nor protein amount of FOXO1 in control and dexa-treated cells. Consistently, FOXO1 remained cytosolic. The assumption that AKT3 may be sufficient to maintain FOXO1 phosphorylated and inhibited is supported by the finding that the unspecific PKB/AKT inhibitor Akti-1/2 abolished phosphorylation and induced nuclear accumulation of FOXO1. Simultaneous staining of cells for FOXO1 and cleaved caspase-3 confirmed apoptotic cell death without nuclear accumulation of FOXO1. However, Akti-1/2 accelerated dexa-induced apoptosis.

Conclusion: These data suggest that phosphorylation of FOXO1 is maintained in insulin secreting cells even when AKT1 and AKT2 expression are inhibited up to 90 %. Dexa-induced apoptosis does not depend on but is aggravated by activation of FOXO1.

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L-arginine is essential for pancreatic beta cell functional integrity, metabolism and defence from inflammatory challenge

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Background and aims: Glucose is known to stimulate insulin secretion via rapid metabolism and generation of key stimulus-secretion coupling factors including ATP. Specific amino acids are also known to be insulinotropic, utilizing various mechanisms of action. The aim of this study was to determine whether L-arginine (a known insulinotropic amino acid) could promote a shift of β-cell intermediary metabolism favoring glutathione (GSH+GSSG) anti-oxidant responses, stimulus-secretion coupling, and functional integrity. **Materials and methods:** Clonal BRIN-BD11 β-cells and mouse islets were cultured for 24h at various L-arginine concentrations (0 to 1.15mmol L⁻¹) in the absence or presence of a sub-lethal pro-inflammatory cytokine cocktail (IL-1β, TNFα, IFNγ). Cells and Islets were assessed for viability, insulin secretion, GSH, GSSG, glutamate, NO•, superoxide, urea, lactate, and for the consumption of glucose and glutamine. Protein levels of NO synthase (NOS-2), AMPK and the HSP72 were also evaluated.

Results: L-arginine at 1.15 mmol L⁻¹ attenuated the loss of β-cell viability observed in the presence of pro-inflammatory cytokines. L-arginine increased cellular levels of reduced glutathione [from 0.13±0.01 (without arginine) to 0.91±0.14µg/mg protein, in the presence of 1.15mM L-arginine] and glutamate [from 6.42±0.5 (without arginine) to 12.8±0.7µg/mg protein, in the presence of 1.15mM L-arginine] but reduced the GSSG/GSH ratio and glutamate release [from 4.15±0.4 (without arginine) to 2.46±0.4µg/mg protein in the presence of 1.15mM L-arginine]. L-Arginine stimulated glucose consumption in the presence of cytokines [from 15.57±2.35 (without arginine) to 27.6±2µg/mg protein, in the presence of 1.15mM L-arginine] while also stimulating AMPK phosphorylation and HSP72 expression. Pro-inflammatory cytokines reduced, by at least 50%, chronic (24h) insulin secretion, an effect partially attenuated by L-arginine [from 928.3±44.6 (without cytokines) to 425.3±108µg/mg protein/24h, in the presence of cytokine cocktail]. Acute (20min static incubation) insulin secretion was robustly stimulated by L-arginine [from 0.06±0.004 (without arginine) to 0.832±0.03µg/mg protein/20min, in the presence of 1.15mM L-arginine] but this effect was abolished in the presence of cytokines.

Conclusion: L-arginine stimulated β-cell insulin secretion, anti-oxidant and protective responses, with increased functional integrity of β-cells and islets in the presence of pro-inflammatory cytokines. Glucose consumption and intermediary metabolism were increased by L-arginine. These results highlight the importance of L-arginine availability for beta-cells during inflammatory challenge which may be associated with both type 1 and type 2 diabetes.

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Antioxidant and antiapoptotic effects of ZnCl₂ in rat pancreatic islets cultured in low and high glucose concentrations

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Background and aims: During prolonged culture of rat islets, glucose markedly decreases cell apoptosis from 5 to 10 mM and increases it moderately from 10 to 30 mM. A similar asymmetric V-shaped profile was observed for glucose-induced changes in the mRNA levels of oxidative stress response genes such as Metallothionein 1a (Mt1a), a metal-detoxifying protein that also exert antioxidant effects in various cell types. This suggests a possible link between oxidative stress and apoptosis under these conditions. In this study, we tested the effect of ZnCl₂, a potent inducer of metallothionein expression, on oxidative stress and islet cell apoptosis induced by low and high glucose concentrations.

Materials and methods: Male Wistar rat islets were cultured for up to 1 week in serum-free RPMI medium containing 5 g/l BSA and 5, 10, or 30 mM glucose (G5, G10, G30) ± 100 µM ZnCl₂. Islet gene mRNA levels were measured by RTq-PCR. Apoptosis was quantified by measuring islet cell histone-associated DNA fragments (commercial ELISA) and by the percentage of TUNEL-positive beta cells. Thiol/disulfide equilibrium was measured as an indica-

tor of mitochondrial oxidative stress in rat islet cell clusters infected with an adenovirus coding “redox sensitive GFP” targeted to the mitochondria (mt-roGFP). Briefly, cells were cultured overnight in the presence of 10% FBS and G5, G10 or G30 \pm 50 μ M ZnCl₂. After culture, mt-roGFP fluorescence ratio (exc: 400/480 nm, em: 535 nm) was measured for 20 min at the same glucose concentration and normalized to the ratio measured after addition of 10 mM dithiothreitol (full reduction, 0%) followed by 1 mM H₂O₂ (full oxidation, 100%). Results are means \pm SEM for at least 3 experiments. Statistical significance of differences between groups was assessed by 1-way ANOVA followed by a test of Newman-Keuls.

Results: In comparison with G10, culture of islets in G5 or G30 significantly increased Mt1a to Tbp mRNA ratio (~50-fold in G5 and ~15-fold in G30) and apoptosis (~12-fold in G5 and ~2 to 4-fold in G30). Culture of dispersed islet cells in G5 and G30 vs. G10 also significantly increased mt-roGFP oxidation (normalized ratios: from 28 \pm 2% in G10 to 44 \pm 2% in G5; from 24 \pm 2% in G10 to 35 \pm 3% in G30). Treatment of islets with 100 μ M ZnCl₂ strongly increased the expression of Mt1a mRNA and protein levels at all glucose concentrations. These effects were accompanied by parallel reductions in early mt-roGFP oxidation and late beta cell apoptosis. Thus, in islet cell clusters, addition of 50 μ M ZnCl₂ to the culture medium significantly reduced by ~40% the increase in mt-roGFP oxidation induced by G5 (normalized ratio: from 44 \pm 2 to 37 \pm 2%), and by ~23% that induced by G30 (normalized ratio: 35 \pm 3 to 32.5 \pm 2). In whole rat islets cultured for 3 to 7 days, addition of 100 μ M ZnCl₂ significantly reduced by at least 27% the stimulation of beta cell apoptosis induced by G5, and by 40–65% the stimulation of apoptosis by G30.

Conclusion: ZnCl₂ protects beta cells from apoptosis during prolonged culture of rat islets in low and high vs. intermediate glucose concentrations. The mechanism involved might depend on metallothionein expression and reduction of mitochondrial oxidative stress.

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Impact on beta cells of myokines secreted by skeletal muscle of differing insulin sensitivity and fibre type

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Background and aims: Decreased beta-cell functional mass and insulin resistance are hallmarks of type 2 diabetes (T2DM). We have shown crosstalk between skeletal muscle of different insulin sensitivity and beta-cells, suggesting that myokines secreted by insulin-resistant skeletal muscle could contribute towards decreased beta-cell function/mass in T2DM. In obese and T2DM individuals, the distribution of muscle fiber types is shifted toward faster, more glycolytic fibers. Therefore, we explored the potential communication of human skeletal muscle cells of different fiber type with different insulin sensitivity and pancreatic beta-cells.

Materials and methods: Human skeletal muscle biopsies were collected from vastus lateralis (V), soleus (S) and triceps (T) and cells with different fiber type were cultured. mRNA expression of genes involved in carbohydrate or lipid metabolism and mitochondrial factors were measured by quantitative RT-PCR. Human myotubes where treated for 24h with or without (control) 20 ng/ml TNF- α to induce insulin resistance. Conditioned media from the fiber types (test: TNF- α -CM-S, V, T; control: C-CM-S, V, T) were collected. Sorted rat primary beta-cells were used to test effects of conditioned media for 24h on death (TUNEL), proliferation (BrdU incorporation) and glucose-stimulated insulin secretion. Data are mean \pm SE with statistical significance ($p < .05$) for all indicated differences.

Results: Muscle cells (n=8) isolated from triceps showed higher expression of phosphofructokinase and lactate dehydrogenase (LDH) A whereas LDH B and hexokinase II were highly expressed in soleus and vastus cells. Lipoprotein lipase CD36 and hormone sensitive lipase were predominantly expressed in soleus and vastus muscle cells. Mitochondrial factors were similarly expressed in V, S and T myotubes, with only PPAR gamma more highly expressed in T. CM-S, -V, -T increased GSIS, whereas TNF- α -CM-S, and -V decreased GSIS to a similar extent. Interestingly, TNF- α -CM-T had no impact on GSIS. Beta-cells treated with either TNF- α -CM-S or -V showed increased death (11 \pm 2 and 13 \pm 3.5 fold increased TUNEL⁺ respectively), and decreased proliferation vs. control conditioned media (7.3 \pm 1.7 vs. 1.1 \pm 0.4 and 6.9 \pm 1.2 vs. 0.9 \pm 0.1 % BrdU positive cells, C-CM-S,V vs. TNF- α -CM-S,V). Again, TNF- α -CM-T treatment had no impact on these parameters vs. C-CM-T treatment. Finally, C-CM-S, V, T all increased beta-cell prolifera-

tion (4.7 \pm 0.3 vs 7.3 \pm 0.6 vs. 6.8 \pm 1.2 vs. 8.6 \pm 1.1 % BrdU positive cells respectively).

Conclusion: Taken together these results show that skeletal muscle prepared from biopsies with different fiber composition retain *in-vitro* their *in-vivo* phenotype. Moreover the impact on pancreatic beta cells of human skeletal muscle cells is fiber specific, with both positive and negative effects depending on different insulin sensitivity. The identification of the fiber specific myokines and their molecular targets on beta-cells may lead to new therapeutic strategies for preservation of functional beta-cell mass in T2DM.

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Aminoacidotoxicity, a potential contributor to beta cell dysfunction in type 2 diabetes: studies *in vitro*

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Background and aims: Pancreatic β -cell dysfunction due to elevated glucose and free fatty acids i.e. gluco- and lipotoxicity with increased basal insulin secretion (BIS) and impaired glucose stimulated insulin secretion (GSIS) has been amply demonstrated in type 2 diabetes (T2D). Despite elevated levels of certain amino acids in T2D e.g. leucine, little is known about the chronic effects of amino acids on β -cell function. We investigated the effect of chronically elevated concentrations of leucine on β -cell function, on contents of cholesterol (CH) and triglycerides (TG) in β -cells and on expression of genes related to β -cell metabolism.

Materials and methods: Isolated mouse islets from adult female NMRI mice and clonal β -cell line, INS-1E cells, were incubated with or without addition of 1 - 10 mM leucine. Insulin was analyzed by RIA. ³H-thymidine incorporation was used to monitor INS-1E cell proliferation and DNA synthesis. RT-PCR was performed using an ABI 7500 FAST PCR machine. CH and TG content in INS-1E cells were determined by a CH GHOD-PAP kit and a TG GPO-PAP kit, respectively. Identification of INS-1E cell proteins was performed by MALDI-TOF mass spectrometry.

Results: After 72 h incubation in isolated islets and INS-1E cells, leucine (1-10 mM) significantly increased BIS ($p < 0.05$ -0.01) and decreased GSIS ($p < 0.01$ -0.001) corroborating our hypothesis of the existence of aminoacidotoxicity. The impairment of the β -cell function in INS-1E cells took place concomitantly with alterations in proteins and genes involved in insulin granule transport and trafficking (e.g. CRMP-2 and Ran) and the oxidative phosphorylation pathway (cytochrome c oxidase ($p < 0.001$)). Leucine down-regulated the expression of insulin 1 gene ($p < 0.001$), whereas Pdx1 ($p < 0.001$) and insulin 2 genes ($p < 0.001$) were up-regulated. Leucine (1, 5, and 10 mM) decreased ³H-thymidine incorporation in INS-1E cells ($p < 0.01$ -0.001), indicating a leucine-induced suppression of INS-1E cell proliferation. Proteomic studies showed that leucine (5 mM) increased the autophagy related enzyme, cathepsin D ($p < 0.01$), decreased serine/threonine-protein phosphatase PP1 ($p < 0.05$), which is related to phosphorylation/dephosphorylation states of proteins, and decreased GTP-binding nuclear protein ($p < 0.05$) and increased dihydropyrimidinase related protein-2 ($p < 0.05$), which are related to insulin granule transport/trafficking. Importantly, in INS-1E cells accumulation of CH ($p < 0.05$ -0.01) took place concomitantly with an up-regulation of enzymes involved in CH biosynthesis (e.g. Hmgcs1, Hmgcr, Mvd, Sqle, and Ldlr ($p < 0.001$ for all)), whereas TG content was decreased ($p < 0.001$).

Conclusion: Our findings indicate that chronic exposure to elevated levels of leucine induces β -cell dysfunction (i.e. aminoacidotoxicity) and accumulation of CH, changes in CH metabolism, mitochondrial metabolism and insulin granule transport/trafficking which may contribute to the development of T2D.

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Beta cell protective effects of using 2-aminobicyclo[2.2.1]heptan-2-carboxylic acid (BCH) as the glutamate dehydrogenase (GDH) activator in db/db mice

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Background and aims: Glutamate dehydrogenase (GDH) is mitochondria enzyme that converts glutamate to α -ketoglutarate, and vice versa, playing an

important role in amplification of glucose-stimulated insulin secretion (GSIS). Recently we found that GDH activator, 2-aminobicyclo-heptan-2-carboxylic acid (BCH) had a strong protective effect on the high glucose/palmitate (HG/PA)-induced INS-1 cell death. We initially examined whether BCH restored HG-induced reduction of GSIS, and HG/PA-induced reduction of insulin gene expression. Next, we investigated which death-related signal is affected by BCH. Finally, we studied the effects of BCH on glycemic control and pancreatic β -cell integrity in db/db mice.

Materials and methods: INS-1 cells were incubated with 25 mM glucose and 0.5 mM palmitate in the absence or presence of 10 mM BCH. We evaluated GSIS, insulin gene expression, and DNA fragmentation. An in vivo study was performed, in which seven-week-old diabetic db/db mice were treated with BCH (0.7 g/kg, $n = 10$) and with placebo ($n = 10$) for 6 weeks. After treatment, an intraperitoneal glucose tolerance test and immunohistologic examinations were performed.

Results: Treatment with BCH restored the HG-induced GSIS inhibition and down-regulated insulin gene expression by HG/PA in INS-1 cells. In addition, treatment with BCH significantly decreased HG/PA-induced INS-1 cell death and phosphor-JNK expression. BCH treatment improved glucose tolerance as well as increasing insulin secretion in db/db mice. BCH treatment increased the percentage of insulin-positive β -cell-to-total islet area ($p < 0.05$) and decreased the percentage of β -cell expressing cleaved caspase 3 ($p < 0.05$).

Conclusion: BCH as the activator of GDH improved the glycemic control. This anti-diabetic effect may be associated with improved insulin secretion, and the preservation of islet architecture, and decreased β -cell apoptosis.

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Conclusion: Together, we characterized the kinetic progression of beta-cell death under different conditions and highlighted a novel method for the discovery of compounds that can prevent beta-cell death. Since diabetes results from a deficiency in functional beta-cell mass, high-content screening will assist in the discovery of therapeutics for improving beta-cell survival.

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Live cell tracking of apoptotic and non-apoptotic beta cell death: multi-parameter high-content screening for compounds that prevent beta cell apoptosis

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Background and aims: Diabetes is a debilitating disease caused by an absolute or relative deficiency in functional pancreatic beta-cell mass. In type 1 diabetes, the specific autoimmune destruction of pancreatic beta-cells leads to an almost complete ablation of beta-cell mass. Type 2 diabetes is characterized by insulin resistance and a decrease in insulin secretion due in part to increased beta-cell death. Theoretically, the course of the disease could be reversed by preventing beta-cell death due to the ongoing autoimmune attack and promoting beta-cell growth. The aims of the present study were to characterize the mode of beta-cell death under different conditions related to diabetes (hyperglycaemia, cytokine and fatty acid exposure, nutrient deprivation, ER stress) and identify compounds that can prevent beta-cell death through high-content screening.

Materials and methods: Dispersed mouse islet cells and MIN6 cells were infected with lentiviral particles carrying a FRET reporter that allows for simultaneous assessment of caspase-3-dependent apoptosis and insulin promoter activity in live cells (eBFP2-DEVD-eGFP downstream of the insulin promoter), and pdx1 promoter activity (mRFP). Nuclear morphology and cell number were assessed with Hoechst 33342 DNA dye. Propidium iodide and AlexaFluor647-conjugated AnnexinV were used to assess different stages of cell death. Bright field images allowed for detection of plasma membrane morphological changes. Cells were cultured in 5 or 20 mmol/L glucose media and exposed to either a cytokine cocktail (TNF- α , IL-1 β , IFN- γ), palmitate, thapsigargin, or serum free media. High-content imaging was conducted using Molecular Devices ImageXpress^{MICRO} temperature- and CO₂-controlled instrument. This eight-parameter high-content screening was conducted on MIN6 cells exposed to a cytokine cocktail and treated with the Prestwick library of off-patent drugs.

Results: Both apoptotic and non-apoptotic beta-cell death were observed when MIN6 cells were exposed to a cytokine cocktail. Apoptosis was the most prominent form of cell death, as characterized by caspase-3 activation, nuclear condensation, AnnexinV incorporation, and membrane blebbing, prior to propidium iodide incorporation. Non-apoptotic modes of beta-cell death were also observed when cells were exposed to other cell death inducers. Through multi-parameter high-content screening we discovered compounds that can reduce the level of cytokine induced beta-cell death. Eleven different drugs from a library of 1120 were selected for follow-up hit confirmation, including vitamin D2, D3, B5, vitamin E analogs, and other drugs known to have anti-apoptotic effects in different cell types.

PS 028 GLP-1 secretion

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Synergism by individual macronutrients explains the marked incretin and islet hormone response to mixed meal: exploration of a novel experimental tool, the oral meal tolerance test in mice

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Background and aims: The glucagon-like peptide-1 (GLP-1) and insulin responses to oral glucose have been well characterised. Fatty acids and proteins also stimulate GLP-1 and islet secretion, however, the relative contribution of the macronutrients to the GLP-1 and insulin response to mixed meal is not known. We therefore explored a novel tool - the oral meal tolerance test (MTT) in mice - to evaluate the differential macronutrient impact on GLP-1 and islet responses to mixed meal.

Materials and methods: Fasted and anesthetized C57BL/6J mice were orally gavaged with 1) a mixed meal consisting of glucose, whey protein and peanut oil with a total caloric load of 0.285 kcal or single macronutrients at same caloric load as mixed meal. For comparison, 2) a test with glucose, whey protein or peanut oil was given in the same quantity as in the mixed meal, i.e., 0.17 kcal glucose, 0.055 kcal whey protein or 0.055 kcal peanut oil. Blood was collected before and 15, 30, 60 and 90 minutes after oral challenge for plasma glucose and insulin analysis and before and 5, 10 and 20 minutes after oral challenge for active GLP-1 determination in plasma.

Results: The early (0–15 min) insulin response to mixed meal was significantly higher (2.8 ± 0.3 nM) when compared to isocaloric loads of glucose (1.7 ± 0.3 nM, $P=0.02$), protein (945 ± 112 pM, $P=0.0002$) and peanut oil (227 ± 28 pM, $P=0.00001$). For comparison, the insulin response (0–15 min) to mixed meal was substantially higher (2.5 ± 0.3 nM) than the response to the single macronutrients in meal quantity (887 ± 140 pM for glucose, 118 ± 45 pM for whey protein and 46 ± 27 pM for peanut oil). Early (0–5 min) increase in active GLP-1 was observed after both mixed meal (2.5 ± 0.2 pg/ml) and glucose (2.5 ± 0.3 pg/ml), however sustained by mixed meal only (3 ± 0.4 pg/ml), whereas no increase in active GLP-1 was observed after glucose and fat alone. No change in plasma glucagon levels was detected between mixed meal and single macronutrients.

Conclusion: This novel approach to explore incretin and insulin secretion in mice revealed that the marked early insulin response to mixed meal emanates from a synergistic, rather than an additive effect of the macronutrients and this is most likely caused by increased GLP-1 secretion. Hence, superior to glucose alone, the MTT offers a physiological tool for exploration of incretin and islet hormone secretion in studies on integrative metabolism and drug development.

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In humans, the removal of duodenum increases GLP-1 secretion

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Background and aims: Obesity has reached epidemic proportions and bariatric surgery has emerged as an acceptable treatment for morbid obesity and, recently, as the most effective therapy for type 2 diabetes. The initial assumption was that the mechanism causing this effect was through weight loss. It is becoming evident that the anti-diabetic effect is not entirely explained by weight loss as there is a consistent observation that the improvement of glucose and insulin levels occurs within days after RYGB, clearly before any clinically relevant weight loss. Instead the 'hindgut' proposes that rapid delivery of partially digested nutrients to the distal bowel up-regulates the secretion of incretins such as glucagon-like peptide-1 (GLP-1).

Materials and methods: To examine the actual role of duodenum in regulating GLP-1 secretion, we studied 11 (6 men, 52.8 ± 2.7 years, body mass index: 28.0 ± 5.5 kg/m²) normal glucose tolerant humans before and after duodenum head pancreatectomy with a 240-min meal test, assaying plasma concentration of GLP-1, GIP, glucagon, insulin, C-peptide and glycaemia.

Results: Post-challenge insulin and C-peptide levels were significantly lower after partial pancreatectomy ($P < 0.01$), although the biphasic physiological insulin secretion after the meal was preserved. When compared with the pre-surgery study, the oral meal ingestion elicited an increase in GLP-1 concen-

trations ($P=0.01$) along with a decrease in GIP secretion ($P<0.01$). Interestingly, the greater the increase in the GLP-1 secretion after surgery, the lower was the reduction in insulin ($r=-0.5$; $p=0.02$) and C-peptide ($r=-0.4$; $p=0.03$). The glucose-induced glucagon suppression did not change after surgery, probably as a consequence of the increase in GLP-1 levels.

Conclusion: The removal of duodenum and the subsequent anastomosis with the ileum caused a significant increase in GLP-1 associated with a significant decrease in GIP. We believe that the removal of duodenum may play an important role in the modulation of GLP-1 secretion and that it may help in explaining some of the data emerging for studies of gastric bypass surgery.

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Glucose absorption rate and insulin sensitivity assessment during a mixed tube infusion in the duodenum, jejunum and ileum in insulin resistant, normo-tolerant obese subjects

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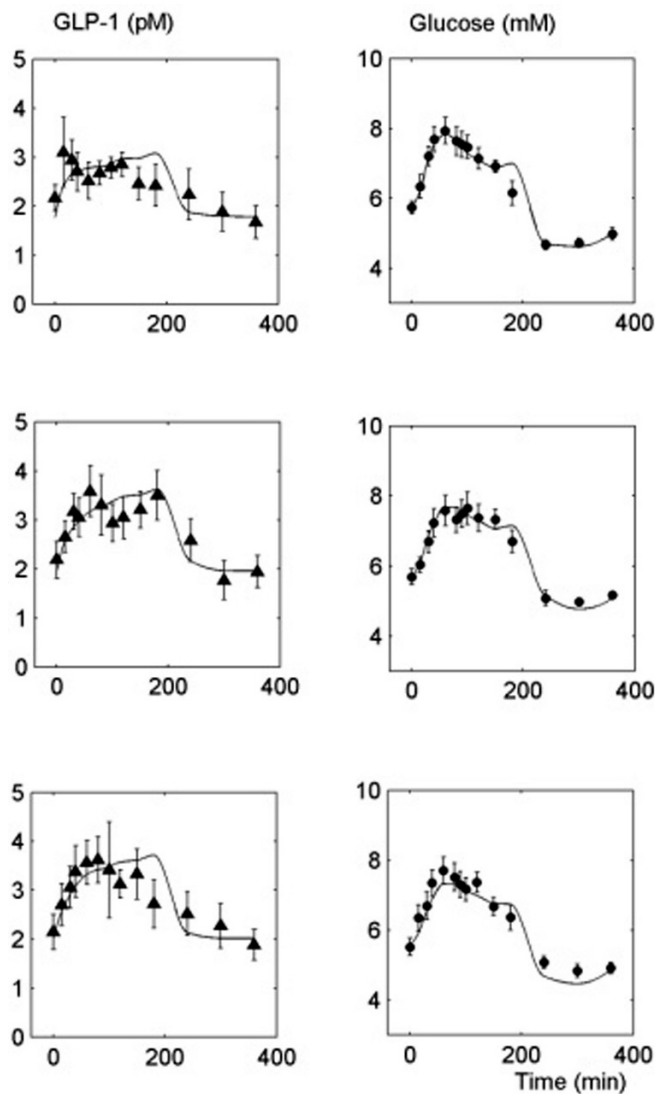
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Background and aims: Duodenal and jejunum bypass as obtained by bariatric surgery results in improvement/resolution of type 2 diabetes (T2D) and improvement of insulin resistance. It has been argued that bypass of the proximal bowel may reduce glucose absorption, which could, at least in theory, improve postprandial glucose homeostasis. Intestinal lipid malabsorption, resulting from the diversion of bilio-pancreatic juices into the terminal ileum, has also been proposed as an alternative hypothesis for the control of diabetes after bilio-pancreatic diversion. However, the mechanisms through which these findings happen are not still elucidated. Aim of the present study is to investigate the differences in insulin sensitivity due to nutrients infusion in different portions of the small intestine, namely duodenum (D), jejunum (J) and ileum (I) in insulin resistant, normo-tolerant (NGT) subjects.

Materials and methods: Ten NGT obese subjects (7 W, 3 M) 45.9 ± 9.1 years old and with a BMI of 39.1 ± 0.7 kg/m² were studied in 3 different occasions. An energy dense tube feed (Nutrison Energy) was infused over 180 minutes in the duodenum, proximal jejunum or ileum and blood drawing was continued over further 180 minutes. Overall, 55.5 g of carbohydrates, 18 g of proteins and 17.4 g of fat were infused. The glucose Ra time course, and the insulin sensitivity were calculated by a validated mathematical model providing an expression for the release of incretin hormones as related to glucose transit into the gut lumen. The model was fitted on the population data (Figure).

Results: The highest glucose absorption rate was observed in the ileum (27.96 ± 5.47 vs 17.60 ± 6.43 D and vs 20.85 J min⁻¹, $P<0.05$). The glucose effectiveness ($SG \times 10^2$) was similar ($P=NS$) in the three intestinal segments (2.80 ± 1.23 D vs 2.73 ± 1.42 J and 3.49 ± 1.22 I). The insulin sensitivity ($SI \times 10^4$) was 40% higher when the test meal was infused in the ileum (0.98 ± 0.42 min⁻¹ pM⁻¹) than in the duodenum (0.55 ± 0.22) and the jejunum (0.68 ± 0.26), $P<0.015$ I vs D.

Conclusion: The delivery of an energy dense tube feed, containing carbohydrates, proteins and lipids, into the ileum enhances glucose absorption simultaneously improving insulin sensitivity.



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Lipid triggered GLP-1 secretion from primary L-cells

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Background and aims: Glucagon-like peptide 1 (GLP-1) is a hormone secreted by enteroendocrine L-cells found scattered along the length of intestinal tract with highest density in the distal ileum and colon. Apart from being a critical regulator of insulin secretion, GLP-1 modulates pancreatic beta-cell proliferation and survival, and mediates satiety. It is not surprising, thus, that GLP-1 based-regimes are already in use in the clinical setting for the treatment of type 2 diabetes. Although GLP-1 is known to be secreted in response to the presence of ingested lipids, the molecular mechanisms involved in the nutrient sensing by L-cells and subsequent secretion of GLP-1 are still unclear. Therefore, understanding these pathways is important for the identification of potentially new therapeutic targets for the treatment of obesity and type 2 diabetes. The aim of this study was to investigate the effects of lipid micelles on GLP-1 secretion by primary L-cells.

Materials and methods: GLP-1 secretion was assayed in primary cultures of murine colonic epithelium. Ratiometric $[Ca^{2+}]_i$ imaging experiments were performed on individual L-cells isolated from transgenic mice expressing the yellow fluorescent protein Venus under the control of the proglucagon promoter. These cells were identified within a mixed colonic culture based on their Venus fluorescence and loading with Fura-2AM. To simulate the conditions epithelial cells experience after a lipid rich meal, "post-prandial micelles",

comprised of oleic acid, 2-monooleoyl glycerol, L- α -lysophosphatidylcholine, cholesterol and taurocholic acid, were applied.

Results: Primary L-cells responded to lipid micelles by secreting enhanced amounts of GLP-1 (24.9 fold stimulation, compared to baseline, $p < 0.001$). The stimulation of GLP-1 secretion by lipid micelles was not attributable to cell lysis, as monitored by lactate dehydrogenase activity released into the supernatant. Fluorescence calcium imaging measurements demonstrated transient elevations in intracellular calcium in response to lipid micelles ($R_{340/380}$ increased 1.23 fold compared to baseline $p < 0.001$ $n=16$). To determine whether these augmented levels of calcium derived from intracellular stores or from extracellular supply, imaging experiments were performed in calcium free saline. It was found that in the absence of extracellular calcium, lipid micelles lack the ability to alter the concentration of intracellular calcium. The modulator of the transient receptor potential (TRP) family, 2-APB, significantly attenuated the secretion of GLP-1, stimulated by lipid micelles (53% attenuation). Similar were the effects of the lanthanides La^{3+} and Gd^{3+} which block calcium-permeable cation channels. Both La^{3+} and Gd^{3+} markedly reduced the secretory responses (51% and 39% respectively, compared to stimulation by micelles).

Conclusion: Lipid micelles stimulate GLP-1 secretion from primary murine cultures. This stimulation of GLP-1 can be blocked by La^{3+} , Gd^{3+} and 2-APB suggesting that activation of non-selective cation/ TRP channels may be involved in the secretory responses by lipid micelles

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High fat diet increases enteroendocrine L cell number in the jejunum of mice in conditions of insulin resistance

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Background and aims: The potent insulinotropic and satiety hormone Glucagon-like Peptide-1 (GLP-1) is released by enteroendocrine L cells which are scattered in the intestinal mucosa. Intriguingly, GLP-1 secretion is diminished in obese patients and in models of obesity in rodents, suggesting that a deficient GLP-1 signal could contribute to the development of obesity. As L cell number can be influenced by nutritional factors such as non-digestible high fructan rich meal, we hypothesized that impaired GLP-1 secretion in obesity could be linked to a decrease in L cell number. The present study aimed to evaluate the number of enteroendocrine L-cells in a diet-induced obesity mouse model.

Materials and methods: 8 week-old mice were fed with either a control diet (4% fat) or a high-fat diet (HFD, 34% fat) for up to 16 weeks. After a diet period, oral glucose tolerance tests (4mg/kg) were performed on 16h-fasted mice. Afterwards, mice were sacrificed and proximal jejunal samples were processed for immunohistochemistry and biochemical investigations. Total enteroendocrine cells and specific L-cells were quantified with chromogranin A and GLP-1 labeling respectively. Two methodological approaches were performed in this study. First, we determined the L cells number (as % of total epithelial cells of tissue) on paraffin-embedded intestinal tissues which were immunostained and quantified by the image J software. A parallel approach was performed on isolated epithelial cells from intestinal tissues, where enteroendocrine cells and specific L-cells were quantified by flow cytometry and results were expressed as % of epithelial cells. In addition, proglucagon mRNA levels were measured in intestinal samples by quantitative PCR.

Results: As expected, mice fed with HFD developed glucose intolerance, hyperinsulinemia and obesity. In jejunum, the number of enteroendocrine cells ($0.22 \pm 0.03\%$) and L-cells ($0.11 \pm 0.01\%$) were identical during all time-course (1 to 12 weeks) analyzed in the study in mice fed with control diet. Surprisingly, HFD doubled the number of enteroendocrine cells ($0.34 \pm 0.02\%$, $p < 0.05$) after 5 weeks of diet and remains high during the 12-week diet period ($0.39 \pm 0.02\%$, $p < 0.01$) when compared to control. In parallel, L cell number increases after 8 weeks of HFD ($0.18 \pm 0.01\%$, $p < 0.01$) when compared to control diet. An increase in L cell number was observed using both methodological approaches. This increase was consistent with enhanced proglucagon expression in proximal and distal intestine.

Conclusion: In this study, we demonstrate that mice fed a HFD have an enhanced enteroendocrine L-cells cell population in the jejunum, which is consistent with a raise in proglucagon expression in the intestine. Therefore, basal impairment of GLP-1 secretion observed in diet-induced obesity cannot be explained by loss of L cells. The nutritional modulation of enteroendocrine cell number could represent an adaptive response to obesity and insulin resistance.

sistance that compensates defective L cell functions downstream hormone synthesis.

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COUP-TFII controls mouse postnatal pancreatic beta cell mass through GLP-1 activated beta-catenin signalling pathway

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Background and aims: The nuclear receptor COUP-TFII (Chicken Ovalbumin Upstream Promotor Transcription Factor, also called NR2F2) has a regulatory role in glucose metabolism. Here, our objective is to identify the role of this transcription factor in pancreatic beta-cell homeostasis.

Materials and methods: Our results are based on transgenic mouse models in which pancreatic beta-cells are made totally deficient in COUP-TFII using the Cre-lox system, on isolated mouse pancreatic islets, and on the INS-1 832-13 beta-cell line with loss of function of COUP-TFII (siRNA) and gain of function of COUP-TFII (overexpression using hCOUP-TFII adenovirus).

Results: Total ablation of the COUP-TFII gene in pancreatic beta-cells of mice results in glucose intolerance and impaired glucose-induced insulin secretion compared to wild type. It also leads to a significant 50% reduction in beta-cell number during the suckling period, when apoptosis and proliferation are essential in establishing the final beta-cell mass. Molecular analysis of pancreatic islets isolated from these mice and of 832/13 INS-1 beta-cells reveal that COUP-TFII up-regulates beta-catenin target genes, such as cyclin D1 and axin-2, by activating beta-catenin gene expression. This suggests that COUP-TFII controls Wnt-beta-catenin signalling pathway, which is known to be implicated in controlling early beta-cell mass development. Glucagon-like peptide-1 (GLP-1) is reported to activate the beta-catenin signalling pathway and its target genes such as cyclin D1. We show that in the absence of COUP-TFII, neither induction by GLP-1 nor its stable agonist exendin-4 can fully activate the cyclin D1 gene. These results demonstrate the requirement of COUP-TFII for GLP-1 activation of the beta-catenin signalling pathway and downstream transcription. Our results suggest that GLP-1, COUP-TFII and the beta-catenin pathway regulate beta-cell mass in a coordinate manner. Given the well established roles of the Wnt/beta-catenin pathway and GLP-1 in beta-cell survival and expansion, these findings may explain why pancreatic beta-cell mass is diminished in mice lacking COUP-TFII.

Conclusion: This work documents an important new role for COUP-TFII in beta-cell biology and links the action of insulinotropic hormone GLP-1, Wnt-beta-catenin and nuclear receptor pathways in determining pancreatic beta-cell mass, which is of direct clinical relevance in diabetes. It has been reported previously that GLP-1 receptor expression is high in pancreatic islets and plasma levels of GLP-1 are high during the suckling period, so our results further highlight a possible role for GLP-1 in controlling beta-cell homeostasis during the neonatal period.

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Glucagon-like peptide-1 secretion from proximal rat intestine

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Background and aims: Glucagon-like peptide-1 (GLP-1) is an incretin hormone, secreted from intestinal L-cells after intake of nutrients. Classically, L-cells are believed to be located primarily in the distal part of the small intestine and the colon. This led to the hypothesis of a duodenal-ileal loop explaining the very rapid GLP-1 response to a meal. Recently, L-cells were found in the proximal small intestine. This study aimed to compare GLP-1 secretion from the proximal and the distal small intestine using the in situ perfused rat intestine.

Materials and methods: Experiments were performed by perfusing either the proximal or distal half of the small intestine in male Wistar rats. Intestinal secretion was stimulated by infusion with the phosphodiesterase inhibitor

3-isobutyl-1-methylxanthine (IBMX; 1 mmol/L). Effluent concentrations of GLP-1 and peptide YY (PYY) were subsequently measured. Furthermore, expression levels of Glucagon and Pyy in the most proximal small intestine were compared to expression levels in the most distal small intestine.

Results: Infusion of IBMX significantly increased GLP-1 secretion from the proximal small intestine from a basal level of 52.9 ± 7.7 $\mu\text{mol}/\text{min}$ to a peak level of 277.5 ± 54.2 $\mu\text{mol}/\text{min}$ ($n=7$; $p=0.0032$). Comparable GLP-1 peak levels were observed from the distal intestine (peak 345.0 ± 18.2 ; $n=7$). However, the overall IBMX response (AUC) was greater in the distal (3156.6 ± 331) compared to the proximal small intestine (1847.4 ± 202.9 ; $p=0.0073$). In contrast, stimulation with IBMX resulted in a significant increase in PYY secretion from the distal intestine from 37.2 ± 3.9 to 175.7 ± 29.0 $\mu\text{mol}/\text{min}$ ($p=0.0021$), but had no effect on PYY secretion from the proximal intestine. Expression analysis showed that expression of Glucagon was only slightly less in the proximal compared to the distal intestine (7.4 fold (95% CI 4.7;11.5) greater in the distal intestine), whereas Pyy expression was largely restricted to the distal intestine (240 fold (125;460) greater relative to the proximal intestine).

Conclusion: These results show that infusion of IBMX stimulates secretion of GLP-1 from both the proximal and distal small intestine, whereas PYY secretion is only stimulated from the distal small intestine. Consequently, GLP-1 secretion is not limited to the distal part of the gastrointestinal tract and the early GLP-1 response could arise from direct stimulation of L-cells in the proximal small intestine.

PS 029 GLP-1 action

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Combination of GLP-1 and FGF-21 causes synergistic weight loss in diet-induced obese mice

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Background and aims: GLP-1 agonism is well-established mono-therapy for diabetes (as an incretin factor causing insulin secretion) and even weight management (as a promoter of satiety). However, nausea is a well known dose limiting side effect associated with GLP-1 agonist therapy. Preclinical data suggests that FGF-21 has potential as a therapeutic agent for diabetes and obesity via promoting insulin sensitization and increasing energy expenditure. In this study, we aimed to understand combination treatment of GLP-1 and FGF-21 in diet-induced obesity by targeting two distinct mechanisms (food intake and energy expenditure) that offers superior glucose and body weight control.

Materials and methods: In DIO mice; 1. To test GLP-1 as a dose response as a continuous subcutaneous infusion at doses of 1, 3, and 10 nmol/kg/day, 2. To test dose response of FGF21 therapy provided as once daily subcutaneous injection (50, 150, and 500 nmol/kg), 3. To test the combination treatment at doses of 3 nmol/kg/day of GLP1 + 50 nmol/kg FGF21.

Results: 1. GLP-1 alone dose dependently caused weight loss resulting in approximately 10% weight loss at highest dose tested. 2. FGF-21 dose dependently lowered body weight reaching 14% weight loss at the highest dose tested. 3. Combination treatment caused 20% weight loss compared to 8% weight loss with GLP-1 at 3 nmol/kg/day or 6% with FGF-21 at 50 nmol/kg.

Conclusion: Combination of GLP-1 with FGF-21 caused synergistic body weight loss in diet-induced obesity.

*p<0.05 versus vehicle+vehicle group; +p<0.05 versus all other groups

Treatment	Cumulative Body Weight Change (g)	Cumulative Food Intake (g)	Fat Mass Change (g)	Fat-free Mass Change (g)	Blood Glucose (mg/dL)
Vehicle + Vehicle (11.4 µl/day + 0.05 ml/10 g)	2.5±0.5	44.1±0.8	0.5±0.5	2.0±0.3	131±6
Vehicle + FGF-21 (11.4 µl/day + 50 nmol/kg)	-0.5±0.5*	42.2±0.7	-2.4±0.4*	1.9±0.2	114±5*
GLP-1 + Vehicle (3 nmol/kg/day + 0.05 ml/10 g)	0.20±0.4*	38.4±1.7*	-1.9±0.2*	2.0±0.3	96±3*
GLP-1 + FGF-21 (3 nmol/kg/day + 50 nmol/kg)	-4.8±0.4**	35.3±1.3*	-4.6±0.4**	0.2±0.2*	85±3*

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Effects of glucagon-like peptide-1 (GLP-1) analogue on fibroblast growth factor-21 signalling pathway in ApoE^{-/-} mice with hypoadiponectinaemia *in vivo*

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Background and aims: Liraglutide is a glucagon-like peptide-1 (GLP-1) analogue that stimulates pancreatic insulin secretion and improves β-cell function. However, it is not clear whether liraglutide achieves its glucose lowering effect only by its known effects or whether other as yet unknown mechanisms are involved. Fibroblast growth factor-21 (FGF-21) is a regulator of insulin action on glucose and lipid metabolism. It is produced predominantly by the liver and, to a lesser extent, by adipose tissue. In this study, we have tested the hypothesis that 1) FGF-21 decreases insulin resistance (IR) and 2) that the glucose lowering effects of liraglutide was, at least in part, due to increased FGF-21 in a new insulin resistant mouse model.

Materials and methods: Sixty four male ApoE^{-/-} mice were randomly divided into two groups for intravenous glucose tolerance tests (IVGTT, n=28) and a hyperinsulinemic-euglycemic clamp study (n=36). Thirty six HFD-fed

ApoE^{-/-} mice were subdivided into four groups. One group was given 100µl (1×10⁹PFU) of adenoviral control vectors (Ad-shGFP, GF group, n= 6). The second group received 100µl of adiponectin RNAi adenovirus (Ad-shAcrp30, ADI group, n= 10). The third group was given 100µl of Ad-shAcrp30 and liraglutide (HEA group, n=10) and the fourth group was given 100µl sterile saline (HF group, n=10). Insulin sensitivity and gene expression were examined by euglycaemic-hyperinsulinaemic clamp and Quantitative real-time PCR, respectively. Plasma insulin, glucose, TG, TC, HDL-C, and LDL-C concentrations were measured.

Results: Hypoadiponectinemia significantly increased FGF-21 mRNA expressions in both liver and adipose tissues and decreased FGF-21 receptor 1 (FGFR-1) and β-Klotho mRNA levels in adipose tissues, as well as FGFR-1-3 and β-Klotho mRNA levels in liver. Treatment with liraglutide markedly increased FGF-21 mRNA expression in liver and FGFR-3 in adipose tissues and restored glucose and lipid metabolism and β-Klotho mRNA expression in adipose tissue as well as FGFR-1, FGFR-2, FGFR-3 and β-Klotho mRNA levels in liver up to the levels observed in ApoE^{-/-} mice fed HFD. Dot blot analyses revealed a marked increase in FGF-21 protein levels in liver, adipose tissue, and plasma of ApoE^{-/-} mice with hypoadiponectinemia. Treatment with liraglutide further increased these proteins in liver and plasma.

Conclusion: We investigated the impact of liraglutide on hypoadiponectinemia induced IR. These data suggested that 1) liraglutide might tissue specifically activate FGF21 signaling in adipose tissue through the β-Klotho-FGFR3 complex while in liver through βKlotho-FGFR1-3 complex, 2) hypoadiponectinemia in ApoE^{-/-} mice fed HFD, exacerbated insulin resistance and resulted in FGF-21 resistance, and 3) Liraglutide completely reversed the insulin and FGF-21 resistance induced by hypoadiponectinemia.

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The effects of acute exercise on insulin secretion in type 2 diabetes

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Background and aims: Type 2 diabetes mellitus (T2DM) is an increasing public health problem and so understanding its pathogenesis is crucial. Recent studies have demonstrated that exercise and diet interventions may improve β-cell function in patients with T2DM, possibly by a mechanism related to incretin hormones. This suggests that pancreatic β-cell dysfunction is potentially treatable with a lifestyle-intervention, however the exercise effect on β-cell sensitivity to different insulin secretagogues has not been studied. The aim of the present study was to investigate whether a single bout of exercise can affect β-cell insulin secretion capacity, in response to glucose, the incretin hormone GLP-1, and arginine.

Materials and methods: Individuals with T2DM were screened and underwent measures of body composition and maximal aerobic capacity (VO₂max). Subjects withheld their oral antidiabetic medication for 7 days prior to testing. No subject was being treated with insulin. The final study population included 8 elder, overweight, T2DM subjects (age 60±6 y, BMI 30.2±5.2 kg/m², VO₂max 24.6±4.3 mL/kg/min, fasting glucose levels 7.06±1.9 mmol/l, 2h OGTT glucose value 15.1±4.5 mmol/l, HbA_{1c} 6.5±0.3%). Before and the morning following a one-hour treadmill walk at 65% of maximum heart rate, a hyperglycemic (5.4 mM above basal) clamp was performed combined with a sequential 0.5 pmol/kg/min primed infusion of GLP-1⁷⁻³⁶ amide and a 5 g bolus injection of arginine hydrochloride. Arterialised blood glucose was measured every 5 min throughout and corrections to the glucose infusion rates (GIR; mg/kg/min) were based on a computational algorithm. Insulin secretion rates (ISR; pmol/min) in response to glucose, GLP-1 and arginine were deconvoluted from peripheral blood C-peptide concentrations using standard parameters for C-peptide clearance. Paired T-tests were used to compare pre/post exercise variables. All data are presented as mean±SD.

Results: Exercise did not alter fasting glucose levels (7.7±1.8 vs. 7.9±2.2 mmol/l, P>0.05). Steady state clamp glucose levels were 12.6±0.2 mmol/l before and after exercise, with a CV% of 2.96±1.4 and 4.3±2.8 respectively. The GIR required to maintain hyperglycemia did not differ between trials (P>0.05). However, glucose-stimulated ISR was reduced after exercise (650.3±113.3 vs 593.0±87.6 pmol/min, P=0.05), while GLP-1 (1380±249 vs. 1493±255, P=0.16) and arginine-stimulated ISR (2371±553 vs 2578±673, P=0.01) were increased after exercise.

Conclusion: The exercise effect on insulin secretion from pancreatic β-cells differed between the stimulus from glucose, GLP-1 and arginine. The exercise

related improvement in insulin secretion in response to GLP-1 and arginine could indicate an enhanced insulin secretion capacity of the β -cells. However, further studies are needed to clarify the mechanisms behind this exercise-induced improvement in β -cell function related to insulin secretagogues.

Clinical Trial Registration Number: H-3-2010-127

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Sustained Exendin-4 secretion through gene therapy targeting salivary glands in Zucker fa/fa rats and high fat diet mice

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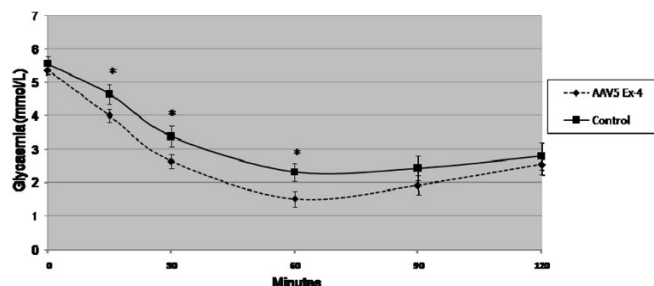
Background and aims: Exendin-4 (Ex-4) is a GLP-1 receptor agonist approved for the treatment of Type 2 Diabetes (T2DM). The aim of the study was to characterize the effects of Ex-4 expressed continuously from salivary glands (SG), following adeno-associated virus-mediated (AAV) gene therapy in two different model of obesity and T2DM. Several trials support an AAV good safety and little toxicity profile. Serotype 5 (AAV5) presented enhanced gene transfer activity in SG. SG exhibit efficient protein secretion into the bloodstream.

Materials and methods: A recombinant AAV vector was produced using a four-plasmid procedure. In accordance with the European Directive (86/609/EEC) and Italian National Health Institute approval, following 5x10¹² DRP/ml vector (encoding Ex-4 or empty) administration, efficacy and metabolic effects in high fat diet (HFD) fed mice (n=20) and Zucker fa/fa rats (n=10) were evaluated.

Results: Ex-4 levels averaged 138.9 \pm 42.3 pmol/L at day 42 in treated mice and 238.2 \pm 72 pmol/L at day 30 increasing to 3 nmol/L at day 60 in treated rats. Transduction specificity was confirmed through a qPCR amplification using specific primer in SG, liver, spleen and pancreas. Treated animals reached a significantly lower weight gain in comparison to controls at day 42 in mice (16.5 \pm 2.7 vs 19.5 \pm 1.9 g; p<0.05) and by day 35 in rats (156.8 \pm 17.5 vs 186.2 \pm 18.6 g, p<0.05) through the study. Transient reduction on food intake was reported. Furthermore, treated mice presented: significant lower leptin circulating levels (2.24 \pm 0.39 vs 5.89 \pm 1.07 ng/ml; p<0.01) and visceral adipose mRNA expression (3.43 \pm 0.48 vs 8.28 \pm 0.72 Arbitrary Unit; p<0.01) and at day 41, a greater insulin-induced reduction in glycaemia during an intraperitoneal insulin tolerance test (Figure 1). A significant reduction in HbA1c (4.7 \pm 0.1 vs 4.9 \pm 0.1 %; p<0.05) and glycosuria (4 cases vs 0) was reported in treated rats.

Conclusion: This study suggests an alternative approach delivering Ex-4 in the treatment of T2DM.

Figure 1. ITT test at day 41, in High Fat-Diet mice (n=20). The graphs represent the average glycaemic values (mmol/L) \pm Standard Error (SE). * = p<0.05.



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Differential effect of pioglitazone, exenatide and combination of pioglitazone and exenatide on adipocyte insulin resistance in type 2 diabetes

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Background and aims: Adipose tissue insulin resistance is characterized by an increased rate of lipolysis and elevated circulating non-esterified fatty acid (NEFA) levels in obesity and type 2 diabetes (T2DM). Pioglitazone improves glycemic control by increasing insulin sensitivity in target organs. Exenatide lowers glucose levels by enhancement of incretin effect. We sought to examine the effect of Pioglitazone (PIO) and combined therapy with PIO and Exenatide (EXE) on adipocyte insulin resistance in T2DM and its association with changes in peripheral insulin sensitivity and beta cell function.

Materials and methods: We studied 30 T2DM subjects (age=55 \pm 37 yrs; BMI=34 \pm 5; FPG=175 \pm 40 mg/dl; HbA1c= 8.3 \pm 1%) who were randomized to receive: (i) EXE (10ug bid, n=11), (ii) PIO (45 mg/d, n=10), or (iii) PIO+EXE (n=9) for 24 weeks. Before and 24 weeks after treatment subjects received an OGTT and two-step hyperglycemic (+125 and +400mg/dl) clamp followed by IV arginine (5g) bolus. The Matsuda Index (MI) of insulin sensitivity and Disposition Index ($\Delta I/\Delta G_{0-120} \times MI$) was calculated from OGTT. Adipocyte insulin resistance index (AdipoIRI) was calculated as the fasting NEFA \times fasting plasma insulin.

Results: PIO + EXE caused a greater reduction in FPG, 2-h glucose and HbA1c compared to PIO alone or EXE alone (all p<0.05). In all groups, change (Δ) in AdipoIRI correlated significantly with Δ HbA1c (r=0.419, p=0.02), Δ MI (r=-0.380, p=0.039) and Δ NEFA (r=0.477, p=0.008) with PIO improved MI of insulin sensitivity from 4.4 \pm 1 to 7.7 \pm 0.9 (p<0.05). Combined PIO + EXE therapy increased MI from 3.2 \pm 0.7 to 6.4 \pm 0.8 (p<0.05) and Disposition Index (DI= $\Delta I/\Delta G_{0-120} \times MI$) from 0.29 \pm 0.06 to 1.1 \pm 0.33 (p<0.05). PIO decreased fasting NEFA (0.648 \pm 0.05 to 0.376 \pm 0.06 μ M, p=0.001), 2-hour NEFA (0.252 \pm 0.09 to 0.09 \pm 0.01 μ M, p=0.009) and the basal adipocyte insulin resistance index (7.35 \pm 2.1 to 1.6 \pm 0.3, p<0.05). No significant changes in plasma FFA concentrations or were observed in patients treated with EXE or a PIO+EXE. The change in MI correlated strongly with Δ AdipoIRI (r=-0.380, p<0.05). Δ DI also correlated with both AdipoIRI (r=0.573, p=0.02) and Δ AdipoIRI (r=0.726, p<0.001). In all groups, changes in insulin secretion in every step during the hyperglycemic clamp were inversely correlated with AdipoIRI at study end (AIR₀₋₁₀₇ r=-0.377 p=0.04, AUC_{INS12-160} r=-0.615, p<0.0001, AUC_{ARG160-190} r=-0.410 p=0.02). At study end, degree of glucagon suppression correlated with AdipoIRI.

Conclusion: PIO alone improved AdipoIRI. Improvement in adipocyte insulin sensitivity could represent a fundamental mechanism by which PIO improves insulin sensitivity. EXE had no effect on adipocyte insulin resistance and attenuated the effect of PIO on AdipoIRI.

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Continuous exenatide infusion markedly improves insulin sensitivity and disposition index in partially pancreatectomised baboons

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Background and aims: Hyperglycemia in Type 2 diabetes (T2DM) is determined by β -cell failure to compensate for insulin resistance. The GLP-1 analogue, Exenatide (EXE) improves glycemic control in T2DM by enhancing insulin secretion. However, the effect of continuous chronic EXE infusion on α and β -cell function has not been well studied previously.

Materials and methods: We examined the effect of a continuous IV infusion (via tether system) of: i) EXE (0.014 μ g/kg.h, n=12) or ii) saline (SAL, n=12) on α and β -cell function in non-diabetic baboons (NDB). At baseline, baboons received a two-step Hyperglycemic (+125 and +225 mg/dl) Clamp followed by an IV Arginine bolus (0.15 g/kg) (HC+A), with a total duration

of 210 minutes. Immediately after HC+A baboons underwent a partial pancreatectomy (~30% total mass) and started EXE or SAL infusion for 13 weeks. At the end of treatment, EXE infusion was stopped for 72 hours before HC+A was repeated and remnant pancreas was collected. In these experimental conditions, the chronic *in vivo* effects of EXE on insulin secretion and insulin action can be evaluated, because of the extensive wash-out period.

Results: Insulin sensitivity (IS) was measured as the glucose infusion rate per unit of insulin (M/I) during steady state conditions, and insulin secretory rate (ISR) was calculated by deconvoluting C-Peptide during the clamp. β -cell function (Disposition Index=DI) was calculated as $ISR \times M/I$, α -cell function as % suppression of glucagon secretion from baseline. Body composition was evaluated by DXA. There was no significant change in food consumption (EXE 55.2 ± 4 , SAL 53.6 ± 2.6 kcal/kg body weight/day). A significantly decrease in body weight (EXE 18.24 ± 0.81 to 17.23 ± 0.65 p=0.034, SAL 18.49 ± 0.62 to 16.67 ± 0.7 kg p=0.006), fat mass (EXE 1.54 ± 0.5 to 0.93 ± 0.2 p=0.044, SAL 1.29 ± 0.2 to 0.86 ± 0.2 kg p=0.035), and lean mass (EXE 15.65 ± 0.4 to 15.22 ± 0.5 p=ns, SAL 16 ± 0.5 to 15 ± 0.6 kg p=0.033) were observed. M/I increased by ~68% after EXE (17.08 ± 3.1 to 28.87 ± 4.2 , p<0.0093) and decreased by 19% in SAL (33.24 ± 9.16 to 26.81 ± 4.41). ISR increased significantly in SAL group (Basal-ISR 2.0 ± 0.3 [Pre] to 4.3 ± 1.0 [Post] pmol/min/g pancreas, p<0.04, Total-ISR 5.6 ± 0.4 to 7.7 ± 1.1 nmol/g pancreas, p=0.08) but not in EXE (Basal-ISR 3.0 ± 0.7 to 4.5 ± 1.1 pmol/min/g pancreas; Total-ISR 7.2 ± 1.0 to 8.0 ± 1.6 nmol/g pancreas, p=ns). DI increased significantly after EXE (1.0 ± 0.1 to 2.2 ± 0.3 , p<0.01), but not after SAL (1.5 ± 0.2 to 1.7 ± 0.3 , p=ns). No differences were found in glucagon suppression or insulin/glucagon ratio between groups (EXE 26.5 ± 3.3 to 22.1 ± 5.2 vs. SAL 25.0 ± 5.5 to 20.2 ± 2.4 mU/l/(ng/ml)⁻¹).

Conclusion: Continuous IV treatment with EXE increased IS and DI, whereas SAL treated NDB had a compensatory increase in ISR, after partial pancreatectomy. In conclusion, chronic Exenatide treatment causes an unexpected marked improvement of IS, with no significant change in insulin secretion, thereby possibly inducing β -cell “rest”.

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Liraglutide attenuated hypoadiponectinaemia-induced deterioration in peripheral and hepatic insulin sensitivity and alterations of gene expression in glucose and lipid metabolism

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Background and aims: Liraglutide is a glucagon-like peptide-1 (GLP-1) analogue that reduces blood glucose levels, increases insulin secretion, and improves insulin sensitivity through mechanisms have not been completely understood. However, the physiological role of liraglutide in glucose and lipid metabolism and relevant signal transduction pathways still need to be determined. We aimed to evaluate the metabolic impact and underlying mechanisms of liraglutide in a hypoadiponectinemia and high fat diet (HFD)-induced insulin resistance (IR) model.

Materials and methods: Sixty four male ApoE^{-/-} mice were randomly divided into two groups for intravenous glucose tolerance tests (IVGTT) and a hyperinsulinemic-euglycemic clamp study. Thirty six HFD-fed ApoE^{-/-} mice were subdivided into four groups: GF group (100 μ l (1 \times 10⁹ PFU) of adenoviral control vectors), ADI group (100 μ l of adiponectin RNAi adenovirus), HEA group (100 μ l of Ad-shAcrp30 and liraglutide) and HF group (100 μ l sterile saline). Insulin sensitivity and gene expression were examined and Quantitative real-time PCR, respectively.

Results: Treatment with Ad-shAcrp30 achieved a 76% reduction of Acrp30 expression in adipose tissues and a 35% reduction of plasma Acrp30 level in the ADI group than in HEA, HF or GF groups. Fasting plasma insulin, FFA, TG and TC were significantly increased in the ADI group. Liraglutide treatment significantly decreased body weight and fasting blood glucose compared with the other three groups. Plasma FFA, TC and TG were significantly suppressed during the clamp procedures in four groups, but remained higher in the ADI group than in the other three groups. The ADI group required a much lower glucose infusion rate (GIR) than the HF and GF mice did during the clamp procedure. The liraglutide treatment markedly increased the GIR in the HEA mice to levels similar to those observed in HF and GF mice. Hepatic PEPCK mRNA expression was significantly up-regulated, while GLUT-1 mRNA expression was significantly down-regulated in ADI group oppose to those three groups. The mRNA expressions of HSL and PPAR γ in adipose tissues were significantly down-regulated in ADI group. The administration

of the Acrp30 siRNA-expressing vector significantly up-regulated the FAS mRNA expression of adipose tissue. However, treatment of liraglutide restored it, and down-regulated ACC and SCD1 mRNA expressions.

Conclusion: This study is the first to reveal the profound anti-insulin resistance effect of liraglutide when administered systemically in murine models of hypoadiponectinemia and HFD. Our data clearly indicate that liraglutide's effects are, at least in part, likely to be mediated by the alterations of gene expressions involved in glucose and lipid metabolism. However, it will be important in future studies to determine the role of liraglutide in the metabolic regulatory pathways of these genes.

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Early DPP-4 inhibition suppresses the progression of diabetes in low-dose STZ mice via alleviation of beta cell death and alpha cell proliferation

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Background and aims: Incretins such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) not only stimulate insulin secretion in glucose-dependent manner but also promote β -cell proliferation and survival, and inhibit β -cell apoptosis. Dipeptidyl peptidase-4 (DPP-4) inhibitor is a new anti-diabetic treatment via blocking incretin inactivation by DPP-4. DPP-4 inhibitors have been reported to be able to delay or suppress the progression of diabetes in some spontaneous animal models, but the mechanism is not well elucidated. First, we confirmed whether DPP-4 inhibition suppresses the progression of diabetes in low-dose streptozotocin (STZ) induced diabetic mice, and then investigated how DPP-4 inhibition affects islet function and morphology.

Materials and methods: Des-F-sitagliptin (Sita), an analog of sitagliptin was administered orally to mice before and after STZ treatment (50 mg/kg/day, 5 consecutive days). We observed for additional 20 days after STZ treatment with continuous administration of Sita. We measured metabolic variables, examined immunohistochemical analysis, and quantified pancreatic hormone contents.

Results: Glycated haemoglobin levels were significantly lower in Sita-treated STZ mice compared to non-treated STZ mice (non-treated: $5.6 \pm 0.2\%$, Sita-treated: $3.9 \pm 0.2\%$, control: $3.7 \pm 0.1\%$). Glucose-stimulated insulin secretion was also significantly higher in Sita-treated mice compared to non-treated mice. In non-treated STZ mice, we observed marked reduction of β -cells with reduction of pancreatic insulin content and huge expansion of α -cells with increase of glucagon content. While in Sita-treated mice, these morphological changes were mild and the islet architecture was preserved better, and reduction of insulin content was not remarkable and increase of glucagon content was not observed. These findings were more striking in mice with administered Sita earlier and longer. To elucidate the effect of Sita on islets, we examined immunohistochemical analysis 24 hr after final STZ injection. TUNEL positive β -cells and caspase-3 positive β -cells were increased in non-treated mice, whereas these apoptotic cells were significantly reduced in Sita-treated mice, equivalently to GLP-1 mimetic exendin-4 treated mice. These findings suggested that Sita suppresses β -cell apoptosis in STZ mice. Interestingly, PCNA positive α -cells were observed in non-treated mice immediately after STZ injection, but the α -cell proliferation was not observed in Sita-treated mice. It is suggested that DPP-4 inhibition can suppress absolute α -cell proliferation observed in non-treated STZ mice. To confirm incretin effects on β -cell protection *in vivo*, we further examined *in vitro* studies using MIN6 β -cell line. STZ induced apoptosis in MIN6 cell dose-dependently. GIP and GLP-1 equivalently suppressed MIN6 cell apoptosis induced by STZ. Intriguingly, GIP and GLP-1 suppressed synergistically MIN6 cell apoptosis by high-dose STZ treatment.

Conclusion: Our present study indicates that early DPP-4 inhibition can significantly suppress the progression of diabetes via alleviation of not only β -cell death but also α -cell proliferation in STZ mice.

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Mechanism of the preventive effect of sitagliptin upon changes induced by short-term fructose administration in liver glucokinase activity

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Background and aims: While the effect of exendin-4 (GLP-1R agonist) and DPP-IV inhibitors upon islet hormones secretion, β -cell mass, appetite and gastric emptying delay has been widely reported, little is known about their effect upon liver glucokinase (GK-I) activity and their physiologic modulators. The aim of this study was to evaluate the effect of exendin-4 and sitagliptin upon fructose (Fr)-induced changes in GK-I activity and the regulatory mechanism of such activity (genic and protein level, cellular compartmentation and interaction with PFK2).

Materials and methods: Adult male Wistar rats received (3 weeks) a standard commercial diet, without (C) or with 10% Fr (w/v) in the drinking water (F). C and F were daily treated either with sitagliptin (115 mg/day per rat) (CS and FS) or exendin-4 (0.35 nmol/kg of body weight) (CE and FE). After such treatment, we measured: 1) Serum glucose (G) (enzymatic method), insulin (I) (RIA) and triglyceride (TG) (enzymatic method) levels; 2) GK-I activity in both cytosolic (CF) and nuclear (DNF) fractions (enzyme-coupled photometric assay), 3) GK-I and PFK2 mRNA (qPCR) and 4) protein GK-I and PFK2 concentration (Western blot [Wb]).

Results:

	C	CE	CS	F	FE	FS
G (mM)	4.5 \pm 0.1	4.2 \pm 0.1	4.5 \pm 0.2	4.9 \pm 0.2	4.2 \pm 0.2	4.7 \pm 0.3
TG (mM)	0.47 \pm 0.01	0.48 \pm 0.06	0.63 \pm 0.09	1.14 \pm 0.12 $\dagger\dagger$	0.72 \pm 0.09*	0.67 \pm 0.05**
I (ng/ml)	0.30 \pm 0.02	0.25 \pm 0.03	0.27 \pm 0.03	1.09 \pm 0.28 \dagger	0.30 \pm 0.05*	0.31 \pm 0.05*

Values are means \pm SEM (n = 20). TG: $\dagger\dagger$ F vs. C, $P < 0.0001$; * FE vs. F, $P < 0.003$; ** FS vs. F, $P < 0.001$. I: \dagger F vs. C, $P < 0.02$; * FE or FS vs. F, $P < 0.03$.

F induced a significant increase of total GK-I activity that was prevented by administration of either exendin-4 or sitagliptin. Most of the GK-I activity was measured in the DNF in C rats but in the CF in F rats. Exendin-4 and sitagliptin administration to F rats prevented such compartmentation changes. GK-I mRNA concentration was significantly higher in F rats and both exendin-4 and sitagliptin administration turn down these increase to values comparable to those measured in C rats. PFK2 mRNA concentration undergo similar changes. GK-I and PFK2 protein expression (Wb) were significantly higher in F rats. While exendin-4 and sitagliptin administration to F did not modify GK-I protein concentration, it induced a significant decrease in PFK2 concentration, suggesting that their effect on GK-I activity would be associated to the latter action.

Conclusion: F administration to normal rats induced a) significant increase of serum TG and I levels but not of fasting blood G; administration of either exendin-4 or sitagliptin to F prevented the development of such changes; b) the significant increase in GK-I activity measured in F would result from a combination of an increased protein synthesis, a switch in its cellular compartmentation towards the cytosol, and an increase in PFK2 protein level. Exendin-4/sitagliptin administration prevented the F-induced changes in GK-I activity by affecting PFK2 concentration and GK-I compartmentation rather than GK-I protein level.

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PS 030 Gastric inhibitory peptide

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Effects of inhibited GIP secretion on high fat diet-induced obesity and insulin secretion

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Background and aims: Gastric inhibitory polypeptide (GIP) is an incretin hormone which is produced by enteroendocrine K cells following nutrient uptake. In conditions of obesity, except for increasing blood glucose concentrations, GIP has a role in compensatory insulin secretion and fat storage in adipocytes, as well as in pancreatic β -cell signaling. However, the extent of GIP effects on insulin secretion on an obese background remains unclear. For that purpose, we have designed GIP-deficient mice and evaluated its effects on insulin secretory capacity and high fat diet-induced obesity.

Materials and methods: We generated knocked-in mice with truncated GIP gene (GIP KI mice). Using immunohistochemistry the intestine of wild type (WT), GIP-deficient heterozygous (GIP^{+/-}) and homozygous (GIP^{-/-}) mice was evaluated. Then, wild type mice (WT), GIP-deficient heterozygous (GIP^{+/-}) and homozygous (GIP^{-/-}) mice underwent oral glucose tolerance test (OGTT) in which blood glucose levels, plasma insulin and total GIP levels were measured. Subsequently, all groups of mice were kept on control fat diet (containing 10% fat) and high fat diet (containing 60% fat) for 8 weeks during which body weight changes were continuously monitored.

Results: Immunohistochemical staining of the small intestine of GIP^{+/-} mice and WT mice revealed GIP-positive cells. In GIP^{-/-} mice GIP-positive cells were not confirmed. During OGTT, GIP^{+/-} mice showed significantly decreased GIP levels compared to WT mice; the total reduction of GIP secretion (AUC-GIP) was 41.7%. In the case of GIP^{-/-} mice, GIP levels were below detectable range. Blood glucose levels in GIP^{+/-} mice and GIP^{-/-} mice showed significant increase compared to WT mice (30 and 60 min after oral glucose load). Insulin levels in the acute phase of OGTT were significantly decreased in GIP^{+/-} and GIP^{-/-} mice compared to WT mice. In conditions of high fat diet, WT mice showed increased weight gain compared to GIP^{+/-} mice and GIP^{-/-} mice; the latter had lowest weight gain among all three groups.

Conclusion: Our mice model of impaired GIP secretion, in which GIP^{+/-} heterozygous mice show reduced GIP secretion while GIP^{-/-} homozygous mice exhibit total lack of GIP secretion, suggests that inhibition of GIP secretion may suppress fat accumulation and insulin secretion in conditions of high fat diet.

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Pasta consumption elicits similar postprandial glucose, but lower GIP and insulin response compared to bread

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Background and aims: Consumption of slowly digestible starch is implicated with a decreased risk for the development of obesity, insulin resistance and type 2 diabetes. Underlying mechanisms of this beneficial effect are not yet elucidated. We aimed to investigate the difference in metabolic response after the consumption of products with slowly and rapidly digestible starch in human subjects.

Materials and methods: Ten healthy male volunteers (age 21.4 \pm 0.5 y, BMI 22.5 \pm 0.6 kg/m² [mean \pm SEM]) participated in a cross-over study, receiving two different meals on separate days. The test meals were fiber-enriched wheat bread and pasta. The products were enriched in ¹³C and the dual isotope technique was applied in order to calculate glucose kinetics: the rate of appearance of exogenous glucose (RaE), endogenous glucose production (EGP), and the glucose clearance rate (GCR), reflecting glucose uptake by tissues. Blood samples were drawn for analysis of total glucose concentration, insulin and incretin concentrations.

Results: The incremental area under the curve (iAUC, 0–2 h) for total glucose concentrations was not significantly different between both meals, however

the iAUC of insulin was significantly lower after pasta ($p < 0.01$). Glucose appearance from pasta was slower compared to bread, resulting in a lower iAUC of RAe compared to bread ($p < 0.0001$) and correlated with low postprandial concentrations of the incretin hormone glucose-dependent insulinotropic polypeptide (GIP). In accordance with the lower insulin concentrations the GCR was lower after pasta, but paradoxically, EGP was more suppressed.

Conclusion: Slower intestinal uptake of glucose from a starchy food product results in lower postprandial GIP and insulin concentrations, but not necessarily in lower glucose concentrations. In view of the proposed effects of GIP on fat storage and resting energy expenditure these results might explain the role of slowly digestible carbohydrates in the prevention of obesity.

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The influence of metabolic syndrome and glucose tolerance on the incretin effect

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Background and aims: Metabolic syndrome (MS) and impaired glucose tolerance (IGT) are high risk conditions for type 2 diabetes, a disease where the incretin plasma levels or biological activity have been shown to be reduced. Previous data indicate that normotolerant (NGT) subjects with the MS have an increased early phase insulin secretion during OGTT, as measured by insulinogenic index (IG30), in comparison to individuals without the metabolic syndrome, independently from insulin resistance. The present study was undertaken to further investigate the alpha and beta cell function and entero-insular axis in these pre-diabetic conditions.

Materials and methods: Using oral (OGTT) and intravenous (IVGTT) glucose tolerance test we studied alpha and beta cell function, insulin resistance, incretin levels and their relationship to impaired glucose tolerance state in 139 subjects with normal fasting glucose, with (59) and without MS (80).

Results: Among normotolerant subjects, MS+ individuals showed in comparison with MS-: higher AUC (0-10) for glucose ($p < 0.05$) but similar first phase insulin secretion ($p = ns$) as measured by Δ AIRG and AUC (0-10) for insulin during the IVGTT; increased ($p = 0.04$) AUC (0-60) for insulin during the IVGTT, although this difference disappeared when HOMA-IR was used as covariate; similar AUC (0-120) for GLP-1 and GIP ($p = ns$) during the OGTT; higher glucagon suppression relative to the increase of glucose ($p = 0.002$) but similar glucagon levels ($p = ns$). Among impaired glucose tolerance subjects, MS+ individuals showed no statistically significant differences in the above parameters. When taken together IGT subjects showed in comparison to NGT individuals: decreased Δ AIRG ($p < 0.01$) and AUC (0-10) for insulin during IVGTT, and these differences remained when HOMA-IR was used as covariate; similar ($p = ns$) AUC (0-60) for insulin during the IVGTT; higher GIP plasma levels and AUC (0-120) for GIP ($p < 0.05$) during the OGTT; similar ($p = ns$) AUC (0-120) for GLP-1 and glucagon levels; lower glucagon suppression relative to the increase of glucose ($p = 0.04$).

Conclusion: In contrast to the data obtained with OGTT, NGT subjects with metabolic syndrome did not show at the IVGTT an increase of early phase insulin secretion. This difference might be due to an increased incretin effect as suggested also by the higher glucagon suppression in relation to the increase of glucose observed in these subjects. IGT subjects try to compensate the impaired beta-cell secretory capacity by a hyper activation of incretin axis as suggested by the increased GIP levels but they fail to restore an adequate insulin secretion and glucagon suppression relative to the glucose rise. Alike we observed the failure of these compensatory mechanisms when the two defects, metabolic syndrome and impaired glucose tolerance, are associated.

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Glucose dependent insulinotropic polypeptide in impaired glucose tolerance and its association with insulin secretion and sensitivity

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Background and aims: Both insulin secretory defect and insulin resistance are present in Bangladeshi T2DM subjects, but the secretory defect seems to have a predominant role. In case of prediabetes, IFG and IGT seem to be

separate disorders where β -cell dysfunction is predominant in IFG and insulin resistance has a major role in IGT. A combined IFG-IGT group has both the defects. Although abnormalities of incretin effects have been established as major determinants of insulin secretion and sensitivity, these have not yet been studied in any Bangladeshi population. As study in prediabetic subjects can give substantial insight on the natural history of the disorders, the present study was undertaken to investigate the association of GIP with glycemic and insulinemic status in IGT subjects.

Materials and methods: The analytic observational study was conducted under a case-control design with age- and BMI-matched IGT subjects ($n = 51$, age 40 ± 6 yrs, BMI 24 ± 2.9) and Controls ($n = 47$, age 41 ± 5 yrs, BMI 29.0 ± 3.5). IGT was diagnosed following the WHO Study Group Criteria. Serum C-peptide and serum glucose dependent insulinotropic hormone (GIP) were measured by ELISA method. Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were calculated by homeostasis model assessment.

Results: Fasting C-peptide value of IGT subjects was not significantly different from the value of the Controls [(ng/ml), Median (Range) $0.68(0.21-1.39)$ vs $0.64(0.18-1.62)$, $p = 0.914$]. Insulin sensitivity was significantly lower in IGT subjects [HOMA%S, $65(22-222)$ vs $71(27-247)$, $p = 0.005$]. Fasting GIP was significantly higher in case of IGT subjects compared to controls [pgm/ml, $74.21(10-190)$ and $49.62(6.1-278)$; ($p = 0.001$)], but postprandial GIP value was not significantly different. The ratio of the postprandial and fasting GIP was significantly lower in case of IGT compared to controls [PGIP: FGIP, $3.47(0.98-22.0)$ and $5.14(0.96-19.85)$, ($p < 0.001$)]. Serum fasting C-peptide and fasting GIP ratio was significantly lower in IGT subjects than that of control subjects [FC-pep: FGIP, $0.037(0.009-0.251)$ and $0.045(0.009-0.205)$, ($p = 0.05$)]. Serum fasting GIP and fasting glucose ratio in Control and IGT were $2.26(0.29-11.26)$ and $3.53(0.41-7.96)$, ($p = 0.002$). A significant positive correlation was found between fasting C-peptide and fasting GIP ($p = 0.019$) in the control subjects. In multiple regression analysis a significant positive association was found between fasting C-peptide and fasting GIP ($p < 0.01$). A significant negative association was found between fasting GIP and HOMA%S ($p = 0.05$). No association was found between fasting glucose and fasting GIP both in simple correlation and multiple regression.

Conclusion: The incretin effect of GIP is diminished in IGT and it is associated with insulin resistance in Bangladeshi T2 diabetic population.

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Secretion and role of glucose-dependent insulinotropic polypeptide (GIP) in metabolic adaptations during pregnancy and lactation in rats

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Background and aims: As well as being a key incretin hormone, glucose-dependent insulinotropic polypeptide (GIP) exerts important regulatory effects on lipid metabolism and energy disposition. Pregnancy and the transition to lactation involve major remodelling of energy homeostasis, yet dynamic changes in intestinal K-cell function are not fully appreciated. The present study investigated changes of GIP synthesis and secretion in the context of metabolic adaptations during pregnancy and lactation in rats.

Materials and methods: Female, virgin, albino Wistar rats (15 weeks old, groups of $n = 6$) were time-mated and caged individually. Pregnancy proceeded without intervention until parturition, at which point litter sizes were standardised to $n = 10$. Food intake, body weight, non-fasting plasma glucose, insulin and GIP concentrations were monitored at 6-8 day intervals during pregnancy and lactation. On day 21 of both pregnancy and lactation an oral glucose (3.2 g/kg b.w. (51.2 kJ/kg)) or oral fat (1.38 g corn oil/kg b.w. (51.2 kJ/kg)) challenge were conducted after an overnight fast, with assessment of plasma glucose, insulin and GIP responses. Small intestines were excised, weighed and processed for measurement of GIP content.

Results: As expected, in pregnant rats body weight increased 1.2-1.5-fold ($p < 0.05$ to $p < 0.001$) from 13 days post coitus, returning to near control levels shortly after parturition. There was also a transient 1.2-fold increase ($p < 0.05$) in body weight on day 12 and 16 of lactation. Energy intake was consistently 1.8-3.4-fold greater in lactating rats compared to controls ($p < 0.01$). Pregnancy also increased energy intake (1.4-fold) on days 14 and 18 post coitus ($p < 0.05$). Pregnant rats exhibited 19.8% decreased circulating glucose levels ($p < 0.01$) accompanied by 1.6-fold elevated insulin concentrations ($p < 0.05$), which rapidly returned to basal levels following parturition. Circulating GIP concentrations (152.3 ± 18.6 pmol/l) were normal during pregnancy but were elevated 1.4-1.9-fold during the lactation period ($p < 0.05$ to $p < 0.01$). In addition, lactating rats exhibited a 58.4% decreased glycaemic excursion after

oral glucose compared to control rats (158.6 ± 14.3 vs. 381.6 ± 38.1 mmol/l.min, respectively; $p < 0.001$). Corresponding glucose-induced plasma insulin and GIP concentrations in lactating rats were not significantly different from controls (72.6 ± 6.3 ng/l.min and 9986.3 ± 1004.2 pmol/l.min, respectively). However, pregnant rats had a similar glycaemic excursion but showed elevated overall insulin levels ($p < 0.001$) accompanied by 32.6% lowered GIP concentrations following the oral glucose challenge ($p < 0.05$). Metabolic responses to an oral fat challenge were similar in control, pregnant and lactating rats. Lactating rats exhibited 1.4-fold elevated intestinal weight compared to pregnant rats ($p < 0.01$), which was also increased by 1.3-fold compared to controls ($p < 0.01$). Assessment of GIP intestinal content revealed pregnant, but not lactating, rats to have 1.5-fold elevated peptide concentrations when compared to controls (654.0 ± 39.2 vs. 167.6 ± 21.4 pmol/g wet weight, respectively, $p < 0.01$).

Conclusion: Metabolic adaptation in pregnancy and lactation are associated with distinct changes of circulating and intestinal GIP concentrations, suggesting a prominent role of intestinal K-cells in partition of energy regulation mediated via extrapancreatic targets.

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Not glucose tolerance but obesity impairs the numerical incretin effect in Japanese subjects

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Background and aims: It is well known that incretin effect contributes to as much as half of insulin secretory response to oral glucose load. Previous studies showed that this effect reduces along with worsening of glucose tolerance and correlates to BMI. These findings are, however, almost based on Caucasian subjects, and little is known on Asian subjects who have different genetic background resulting in much lower insulin secretory capacity. Previously, we have reported for the first time that incretin effect in Asian normal glucose tolerance (NGT) subjects is comparable to that in European subjects. Thus, in this study, we aimed to investigate whether glucose tolerance and BMI influences incretin effect.

Materials and methods: Isoglycemic 3-hour oral (75g oral glucose tolerance test (OGTT)) and intravenous glucose administration was performed in 25 Japanese subjects (age: 38.3 ± 13.6 (23–68) years; body mass index (BMI): 24.0 ± 4.1 (17–33) kg/m² (mean \pm S.D. (range))) who consists of 16 NGT, 4 impaired glucose tolerance (IGT) subjects, and 5 patients with type 2 diabetes (T2DM). In the isoglycemic test, intravenous glucose infusion (20% wt/vol) was performed aiming to reproduce the plasma glucose profile at OGTT using artificial pancreas system STG-22 (Nikkiso, Tokyo, Japan). Plasma or serum levels of glucose, insulin, C-peptide, glucagon, free fatty acids (FFA), gastric inhibitory polypeptide (GIP) and glucagon-like polypeptide-1 (GLP-1) were determined. Incretin effect values were calculated by relating the difference in integrated incremental β -cell secretory responses.

Results: The glucose excursions during OGTT were closely reproduced by intravenous glucose infusion. Plasma concentrations of GIP and GLP-1 increased significantly after oral glucose load but not increased after intravenous glucose load. There was significant negative correlation between the incretin effect and BMI ($r = -0.47$, $P = 0.02$), while there was not significant correlation between the incretin effect and integrated glucose concentration during glucose administration. The numerical incretin effect was not significantly reduced in T2DM (47 ± 12) compared with IGT (47 ± 3) and NGT (51 ± 13). Integrated incremental β -cell secretory responses relative to integrated glucose concentration was remarkably reduced in T2DM. Furthermore, there were significantly negative relationships between β -cell secretory responses and glucose excursions both in oral and intravenous glucose administration similarly ($r = -0.59$, $P < 0.01$ and $r = -0.43$, $P < 0.05$ respectively).

Conclusion: In this study, we show that the numerical incretin effect is impaired in obesity as previously reported, and, however, is not always reduced in T2DM. The severely impaired β -cell response to intravenous glucose administration might be reflected to this finding in Japanese T2DM. Our results suggest that it is necessary to interpret the incretin effect carefully in dealing with the subjects with genetically different β -cell backgrounds.

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Enteral supplementation with glutamine, fibre and oligosaccharide modulates incretin secretion

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Background and aims: Secretion of incretin hormones, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1, are stimulated by oral loading of various nutrient components. Recently, it is reported that glutamine and dietary fibre intake modify incretin secretory profiles in human. Furthermore, short-chain fatty acid brought by dietary fiber and oligosaccharide is suggested to increase plasma incretin concentration. Currently, enteral supplementation product enriched with glutamine, dietary fiber, and oligosaccharide (GFO) is available as prebiotics in clinical situation. Thus, in the present study, we examined the effects of GFO on incretin secretion.

Materials and methods: We recruited 20 healthy volunteers. Oral GFO (including 9 g glutamine, 15 g fiber, 18 g carbohydrates) and glucose (18 g) were administered on separate days. Plasma or serum levels of glucose, insulin, C-peptide, GIP and GLP-1 were determined over 180 min.

Results: Plasma glucose level is higher in Glucose loading than in GFO loading at 30 min ($p < 0.01$) and area under the curve of (AUC-) glucose in glucose loading was significantly higher than in GFO loading ($p < 0.05$). Plasma GLP-1 level is higher in GFO loading than in Glucose loading at 30 min (GFO 26.5 ± 2.4 vs. Glucose 21.1 ± 1.9 (pg/ml), $p < 0.01$), 60 min (22.1 ± 1.8 vs. 12.1 ± 1.4 (pg/ml), $p < 0.01$), and 120 min (16.1 ± 2.2 vs. 12.1 ± 1.7 (pg/ml), $p < 0.01$). AUC-GLP-1 in GFO loading was significantly higher than in Glucose loading ($p < 0.01$). In contrast, plasma GIP level is higher rather in Glucose loading than in GFO loading at 30min (GFO 89.7 ± 5.8 vs. Glucose 126.7 ± 11.1 (pg/ml), $p < 0.01$) and 60min (66.1 ± 3.2 vs. 84.3 ± 4.9 (pg/ml), $p < 0.01$). AUC-GIP in Glucose loading was significantly higher than in GFO loading ($p < 0.01$). Serum insulin and C-peptide levels showed no significant difference at each time point.

Conclusion: These results showed that GFO loading induce more GLP-1 secretion and less GIP secretion compared to glucose loading which elicit higher blood glucose level. It is suggested that GFO has a potential of dietary supplemental intervention to improve glucose metabolism and may have a favorable influence on metabolic status by modulation of incretin signaling.

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The effects of glucose and meal ingestion on incretin secretion in Japanese subjects with normal glucose tolerance

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Background and aims: Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are major incretins and their secretion during various nutrient loads are evaluated well in Caucasians. However, little is known about relationship between incretin secretion and kinds or amounts of nutrition load in Japanese subjects. In the present study, we investigated how incretin levels are associated with the different amounts of glucose load or meal ingestion by measuring plasma GLP-1 and GIP levels after the administration of 17g or 75g glucose or mixed meal in Japanese normal glucose tolerance (NGT) subjects.

Materials and methods: We recruited 10 Japanese healthy volunteers. The subjects had no history of hypertension, hyperlipidemia, or kidney and liver diseases and did not take any drugs 2 weeks before the study. They participated in 75g oral glucose tolerance test (OGTT), 17g OGTT and meal tolerance test (MTT). Plasma glucose (PG), serum insulin (IRI), serum C-peptide (CPR), plasma total GIP, and plasma total GLP-1 during OGTTs and MTT were determined.

Results: Judging by the results of 75g OGTT, all the subjects were diagnosed NGT according to WHO criteria with fasting plasma glucose and 2-h glucose levels below 6.1 and 7.8 mmol/l, respectively. Between OGTT studies, AUC-PG, AUC-IRI and AUC-CPR in 75g OGTT were larger than those in

17g OGTT. Regarding incretins, AUC-GIP was significantly larger in 75g OGTT than in 17g OGTT. On the other hand, AUC-GLP-1 was not significantly different between 75g OGTT and 17g OGTT. Between MTT and 75g OGTT, there was no significant difference in AUC-PG, AUC-IRI, AUC-CPR and AUC-GLP-1. However, AUC-GIP was significantly larger in MTT than in 75g OGTT.

Conclusion: By comparing the results of the three loading tests (75g OGTT, 17g OGTT, MTT), we speculate that AUC-GIP was altered by contents of each loading test while AUC-GLP-1 was not. Our results suggest that nutritional composition might have larger effect on GIP secretion than on GLP-1 secretion in Japanese NGT subjects.

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Elevated early GLP-1 and increased overall GIP response in type 2 diabetes as compared to the general population

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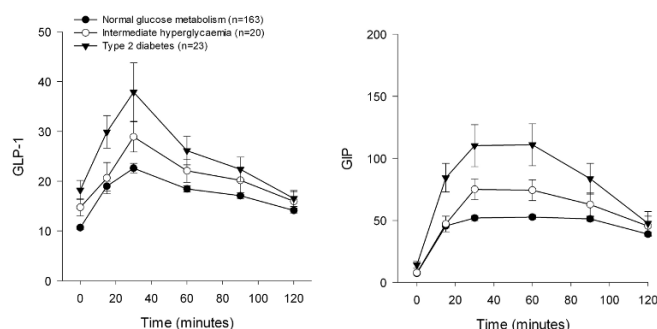
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Background and aims: Therapeutic interventions target enhancement of the gut-derived incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) because of the stimulating effects on insulin secretion. Despite this, assessment of the incretin response in type 2 diabetes has led to conflicting results possibly due to selected control groups. We aimed to assess meal-related and oral glucose related GLP-1 and GIP responses among persons with early type 2 diabetes and intermediate hyperglycaemia as compared to a population-based sample of persons with normal glucose metabolism.

Materials and methods: In a population-based study, 163 persons with normal glucose metabolism, 20 with intermediate hyperglycaemia and 23 with early type 2 diabetes received a standardized mixed meal (75 g carbohydrates, 50 g fat and 24 g proteins) and an oral glucose tolerance test (75 g glucose) on separate occasions. GLP-1 and GIP profiles up to t=120 minutes (oral glucose tolerance test) and t=240 minutes (mixed meal test) were measured in plasma, after extraction. Time-dependent GLP-1 and GIP profiles were analysed by linear mixed models. A significant interaction between time and type 2 diabetes state is interpreted as a stronger rate of increase (or decrease) attributable to type 2 diabetes state.

Results: Among all subgroups, oral glucose elicited a higher GLP-1 ($p<0.05$), but lower GIP ($p<0.05$) response than the mixed meal. As compared to persons with normal glucose metabolism, patients with diabetes had higher fasting levels of GLP-1 ($p<0.01$), but not GIP. Patients with diabetes had higher overall GLP-1 and GIP levels following oral glucose ($p<0.01$) as compared to persons with normal glucose metabolism (Figure). Persons with intermediate hyperglycaemia had intermediate GLP-1 ($p<0.05$) and GIP levels (NS). GLP-1 and GIP responses to the mixed meal showed comparable results (data not shown). More specifically, as compared to persons with normal glucose metabolism, the initial GLP-1 response (t=30) following oral glucose in diabetes patients was stronger (p -value interaction <0.01), followed by stronger suppression later on (t=120, p -value interaction <0.05). The GIP response was more pronounced in diabetes patients (t=15-90 min, p -value interaction <0.01).

Conclusion: We found higher fasting GLP-1 and an elevated early, but abridged GLP-1 response together with an increased overall GIP response among diabetes patients as compared to persons with normal glucose metabolism, from the general population. This suggests a role of GLP-1 and GIP in distinct pathophysiological mechanisms in type 2 diabetes.



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PS 031 Metabolic effects of other hormones

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Sex hormone-binding globuline correlates positively with insulin sensitivity in pubertal girls with type 1 diabetes mellitus

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Background and aims: Low sex hormone binding globuline (SHBG) levels have been associated with insulin resistance and cardiovascular risk in people without diabetes. In adolescents with type 1 diabetes (T1DM) a decrease in insulin sensitivity is observed during puberty and girls are more insulin resistant than boys. The underlying conditions depend largely on endocrine factors, however the relationship between sex hormones and insulin sensitivity measured directly as glucose uptake has not been described in this population. The aim of the study was to assess the relationship between puberty-related hormones and insulin sensitivity in pubertal girls with T1DM.

Materials and methods: The study group consisted of 34 pubertal girls aged 11.3–18.9 years (mean±SD: 15.2±2.3) with T1DM duration of 4.57±3.4 years. Insulin sensitivity (glucose uptake) was expressed as M value in mg/kg/min calculated for the last 30 min of the euglycaemic hyperinsulinaemic clamp. SHBG was measured by solid-phase chemiluminescent assay, dehydroepiandrosterone sulfate (DHEAS) - by radioimmunoassay, estradiol and testosterone - by competitive immunoassay, luteinizing hormone, follicle stimulating hormone and prolactin - by immunometric technique. Correlation coefficient (r) and multivariable regression with backward stepwise variable selection were applied for statistical analysis.

Results: Only two hormones significantly correlated with insulin sensitivity. A significant positive correlation was found between M value and SHBG levels ($r=0.46$, $p=0.008$), this correlation remained significant after correction for potentially confounding factors ($\beta=0.46$, $p=0.004$). A negative correlation was found between DHEAS and M value ($r=-0.43$, $p=0.013$) but it was not statistically significant after correction for confounding factors.

Conclusion: Lower SHBG levels in pubertal girls with T1DM may promote insulin resistance increasing patients' cardiovascular risk.

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Inverse correlation between serum insulin and sex hormone binding globulin in a population survey in the south-west of Sweden

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Background and aims: Obesity is associated with low levels of sex hormone binding globulin (SHBG). While the reason is not fully understood we aimed to study the association between serum insulin and levels of SHBG in a random population.

Material and methods: Between 2001 and 2005 a random sample of 2816 participants aged 30–74 years were enrolled in a cross-sectional survey in the South-west of Sweden. Fasting blood samples were collected and a clinical evaluation was performed. An oral glucose tolerance test (OGTT) was conducted in all subjects without known diabetes. Diabetes mellitus (DM) was defined according to criteria from WHO, and clinical characteristics were used to discriminate between type 1 (T1D) and type 2 diabetes (T2D). Analyses of SHBG were successful in 2761 participants (98%), who thus constituted the current study population.

Results: We found significant inverse associations between levels of SHBG and fasting serum insulin in both genders (men: $\beta=-0.081$, $p<0.001$, women $\beta=-0.079$, $p=0.014$), which were independent of differences in age and BMI. The associations remained when also differences in fasting plasma glucose were accounted for (men: $\beta=-0.061$, $p=0.024$, women $\beta=-0.070$, $p=0.033$). On

the contrary, subjects with T1D exhibited significantly higher levels of SHBG than both subjects with T2D ($\delta=23\text{nmol/L}$ CI 8–38nmol/L $p=0.002$), and non-diabetic subjects ($\delta=15\text{nmol/L}$ CI 1–29nmol/L $p=0.035$).

Conclusion: These findings are consistent with high levels of SHBG in T1D, and correspondingly low levels in T2D subjects, suggesting an inhibitory effect of insulin on the SHBG production in the liver.

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Testosterone, SHBG and the metabolic syndrome: an individual participant data meta-analysis

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Background and aims: Increasing evidence suggests a role for testosterone and SHBG in the etiology of the metabolic syndrome (MetS). We performed a collaborative analysis to determine the strength of these associations overall and in different circumstances and to examine the contribution to individual MetS components.

Materials and methods: Individual data from 16 cross-sectional studies on 3661 men who did have the metabolic syndrome and 9565 men who did not. The metabolic syndrome was defined using the National Cholesterol Education Program-Third Adult Treatment Panel (NCEP-ATPIII) criteria. Odds ratios (ORs) for the metabolic syndrome by quartiles of total testosterone, SHBG and free testosterone were calculated and adjusted for study, age, smoking status, alcohol consumption, physical activity, and additionally for BMI and hormone levels.

Results: Men with low total testosterone, SHBG and free testosterone levels were more likely to have the metabolic syndrome. After adjustment for covariates, the ORs for the highest versus lowest quartile were 5.42 (95% CI 4.77–6.16; $P<0.001$ for continuous linear trend) for total testosterone, 4.66 (95% CI 3.91–5.54; $P<0.001$) for SHBG and 2.97 (95% CI 2.48–3.56, $P<0.001$) for free testosterone. The associations were attenuated but remained significant after further adjustment for BMI and hormone levels (total testosterone: OR_{Q4 vs Q1} 1.63, 95% CI 1.30–2.50, $P<0.001$; SHBG: OR_{Q4 vs Q1} 2.33, 95% CI 1.86–2.91, $P<0.001$ and free testosterone: OR_{Q4 vs Q1} 1.57 (1.27–1.94), $P<0.001$). A significant interaction between BMI and SHBG was found ($P<0.001$). ORs for Q4 versus Q1 of SHBG were 4.65 (95% CI 2.78–7.79) in men with a BMI $<25\text{ kg/m}^2$, 2.94 (95% CI 2.25–3.81) in men with a BMI of 25–30 kg/m^2 , and 2.18 (95% CI 1.46–3.25) in those with a BMI $>30\text{ kg/m}^2$. There were no significant subgroup effects for age, smoking status and other comorbidities. Among the five MetS components, associations were strongest for waist circumference and triglyceride and associations were weakest for blood pressure.

Conclusion: A robust, dose-response relationship exists between testosterone and SHBG levels and odds of the metabolic syndrome in men. The association between SHBG and MetS appears to be stronger in leaner men. The contribution of individual MetS components varies, with testosterone and SHBG being most strongly associated with abdominal obesity and hypertriglyceremia.

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Testosterone: a possible mediator between bone remodelling and energy homeostasis

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Background and aims: Recent findings show that osteocalcin (OCN) a newly identified osteoblast derived mediator of energy balance, not only enhances insulin secretion and insulin sensitivity, but also enhances testosterone production in the Leydig cells of male mice. Low testosterone levels have been linked to metabolic syndrome and increased insulin resistance in men in numerous human studies. Our aim was to assess the relationship between OCN and testosterone levels in normal and impaired glucose tolerant male subjects and the possible role of serum testosterone in the metabolic links of OCN because no human data are available until now.

Materials and methods: 61 obese and non-obese healthy and “prediabetic” male subjects (aged 38.6 ± 13.2 years) were examined who were previously non-treated for glucose intolerance. OGTT, ivGTT and hyperinsulinemic euglycemic clamp were done to assess glucose tolerance, insulin secretion and sensitivity. Full metabolic profiling, adipocytokines, OCN, osteoprotegerin (OPG), nuclear factor κ B ligand (RANKL) and sex steroids were measured. Lean and fat body mass were determined by DEXA.

Results: Based on the OGTT 22 subjects were normal (NGT), and 39 subjects impaired glucose intolerant (GI = IFG/IGT or 2DM). Strong positive bivariate correlation was found between OCN and testosterone levels ($r=0.37$, $p=0.003$) which was independent of age, body mass index (BMI) and body fat percent (BFP). No correlation was observed between OCN and FSH, oestradiol, or dehydroepiandrosterone. We found that the significant correlations observed between OCN and OGTT AUC (glucose area under the curve ($r=-0.4$, $p=0.001$), ivGTT glucose AUC ($r=-0.38$, $p=0.003$), fasting FFA ($r=-0.37$, $p=0.004$), HDL-cholesterol ($R=0.39$, $p=0.002$) were unaffected if data were adjusted with serum testosterone, but disappeared between OCN and clamp measured muscle glucose utilization. Feature selection (variable ranking) analysis showed that serum testosterone was ranked as 24.4/67 (average merit 0.1 ± 0.004) to determine OCN levels, ranked higher than BFP, BMI, M_{fat} , HbA1c%, or OGTT glucose levels but ranked lower than serum leptin, adiponectin, OPG and RANKL.

Conclusion: The connection between OCN and testosterone levels observed in animal models is supported by these human data. We found a strong positive correlation between serum OCN and testosterone levels in men which was not dependent of age and body composition. Our data suggest that in male subjects the antihyperglycemic effect of OCN is regulated not only by adipokines but by serum testosterone levels and OPG and RANKL forming the possible “OCN - insulin” axis.

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Prevention of obesity and insulin resistance by estrogens: dispensable role of the estrogen receptor alpha activation function 1

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Background and aims: Estrogens prevent visceral adiposity, insulin resistance and type 2 diabetes, but their therapeutic potential is strongly limited by their deleterious proliferative action on sexual targets, namely the mammary gland and the uterus. These beneficial metabolic effects result from activation of estrogen receptor alpha ER α which regulates the transcription of many genes through two activation functions (AF-1 and AF-2). We recently demonstrated that, although ER α AF-1 is absolutely required for the uterine proliferative action of estrogens, it is dispensable for their beneficial atheroprotective action of estrogens, paving the way for a selective AF-1-independent ER α modulation devoid of sexual effects. In the present study, we aimed to characterize the involvement of ER α AF-1 domain in the beneficial effects of estrogens on body composition and glucose homeostasis.

Materials and methods: ER α -deficient (ER $\alpha^{-/-}$) AF-1-deficient (AF-1 $^{\Delta}$) female and male mice have been maintained under a chow diet until 7 months of age or submitted to a high-glucose high-fat diet, and compared to their respective littermate wild-type controls in terms of weight gain, body composition and i.p. glucose tolerance test ($n=8-10$ by genotype). Then, to avoid interference with endogenous steroid levels, female mice were ovariectomized at 4 weeks of age and were implanted with subcutaneous pellets allowing a chronic and stable administration of 17 β -estradiol (E2, 80 μ g/kg/d) for 3 months.

Results: As compared to their littermates, female and male ER $\alpha^{-/-}$ mice were both characterized by significantly accelerated weight gain (+36% and +58% for male and female mice on chow diet respectively), massive accumulation of adipose tissue in subcutaneous (+61% and +64% for male and female mice on chow diet respectively) and perigonadic (+62% and +63% for male and female mice on chow diet respectively) sites, hyperinsulinemia and glucose intolerance (AUC: +32% and +35% for male and female mice on chow diet respectively). Similar results were observed under high-fat diet. In striking contrast, weight gain and adiposity constitution were not altered in female and male AF-1 $^{\Delta}$ mice fed either a chow or high-fat diet. Furthermore, glucose tolerance was totally preserved in male AF-1 $^{\Delta}$ mice and modestly impaired in female AF-1 $^{\Delta}$ mice. Since we confirmed that ER $\alpha^{-/-}$ mice exhibit a significant

elevation of endogenous levels of sex steroids, we then studied the effect of chronic E2 administration in ovariectomized female mice on a chow diet. As expected, E2 exerted a strong preventive effect against fat mass accumulation in wild-type mice (-61% subcutaneous and -68% perigonadic, $p<0.001$). This beneficial action on body composition was abolished in ER $\alpha^{-/-}$ mice, but conserved in AF-1 $^{\Delta}$ mice.

Conclusion: As previously demonstrated for the vasculoprotective actions of estrogens, we now provide evidence that ER α -mediated beneficial effects on body composition and glucose tolerance can be obtained in the absence of ER α AF-1 domain. The present study thus reinforces the interest for an AF-1-independent specific modulation of ER α aiming to uncouple the metabolic and vascular protective actions from undesired proliferative effects on reproductive tissues.

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Association of insulin, proinsulin and IGFBP1 with cardiometabolic risk factors and cardiovascular mortality in an elderly population

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Background and aims: Insulin and the insulin-like growth (IGF) systems are phylogenetically closely related. Insulin down-regulate the secretion of insulin-like growth factor binding protein 1 (IGFBP1) from the liver. Previous studies have shown conflicting results regarding the association of circulating insulin, proinsulin and IGFBP1 with cardiometabolic risk factors and mortality. Our aim was to examine the association of insulin, proinsulin and IGFBP1 with cardiovascular (CV) mortality in an elderly population.

Materials and methods: Of all persons aged 70 to 80 years, in a rural Swedish municipality, 852 (73%) accepted to participate in the project. Participants were evaluated by disease history, clinical examination and fasting blood samples including insulin, proinsulin and IGFBP1. They were then followed for 8 years.

Results: Circulating insulin and proinsulin levels were highly correlated at the initial examination and were positively associated with diabetes mellitus, BMI (body mass index), triglycerides but negatively with IGFBP1. IGFBP1 levels were negatively associated with cardiometabolic risk factors as BMI and triglycerides and positively associated with age. At follow up 231 individuals had died, 134 of cardiovascular causes, 40 of malignancies. When insulin, proinsulin and IGFBP1 levels were divided into quintiles, CV mortality was significantly increased for quintile 5 of proinsulin and quintile 4 and 5 of IGFBP1. For insulin a U-shaped relation was found with the lowest CV mortality in quintile 3. When adjusted for cholesterol, BMI, fasting blood glucose, smoking and systolic blood pressure the CV mortality risk remained significant for proinsulin and IGFBP1, but not for insulin.

Conclusion: Our results in an unselected community population of elderly show that proinsulin and IGFBP1 are associated with CV mortality.

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Does amylin affect bone structure in the diabetic state?

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Background and aims: Amylin is co-secreted with insulin from the β -cell, both hormones responding to the same stimuli; it shows some of the effects reported for GLP-1 such as diminishing food intake, slowing gastric emptying and being lipolytic. Amylin also reduces body fat, and is being proposed for the therapy of obesity and diabetes. It has been shown that GLP-1 ameliorates the impaired bone turnover of insulin-resistant (IR) and type 2 diabetic (T2D) models. We have investigated the possible effect of prolonged treatment with amylin on the altered trabecular bone structure in IR and T2D states.

Materials and methods: Male Wistar rats were kept on standard chow and water ad libitum. Normal (N), IR (by fructose) and T2D (streptozotocin at

birth) were 3-day treated with amylin (10^{-9} M) or saline (control), $n=10$ /group, through an osmotic pump subcutaneously implanted. In fed conditions, blood samples were taken before (basal) and by the end of treatment for plasma glucose, insulin (RIA) and amylin (ELISA) measurements; then, rats were sacrificed and the femora were collected, embedded in methylmethacrylate, and sections of the proximal metaphysis were stained with von Kossa's and Goldner's trichrome for the analysis of bone static parameters: bone mineral density (BMD) and mineral content (BMC) -by Lunar PIXImus-, bone volume per total tissue volume ratio (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), erosive surface/bone surface (ES/BS) and osteoclastic surface (OcS/BS) -light microscopy-.

Results: In plasma, basal insulin and glycaemia, as expected, were higher in IR than those in N (both $p<0.05$); in T2D, insulin was lower, and glycaemia, higher (both, $p<0.01$ vs N); amylin did not affect glycaemia in any group, but reduced insulin in IR ($p<0.001$); basal amylin was equal in the three groups (overall mean: 15 ± 3 fmol/ml), and increased to 34 ± 5 fmol/ml during treatment, within the physiological range. In femur, no differences were observed in BMD or BMC in either model vs N (0.162 ± 0.005 mg/cm³ and 0.300 ± 0.014 g, respectively); however, BV/TV and Tb.N were decreased in both ($p<0.01$ vs N), IR (19.1 ± 1.5 % and 1.01 ± 0.09 mm⁻¹, respectively) and T2D (18.8 ± 2.1 % and 1.03 ± 0.09 mm⁻¹), these corresponding values in N being 26.2 ± 1.9 % and 1.67 ± 0.07 mm⁻¹. Conversely, trabecular separation, which was 465 ± 26 μ m in N, showed to be increased to 820 ± 43 μ m and 769 ± 59 μ m in IR and T2D, respectively (both, $p<0.01$); no decrease in Tb.Th was observed in the latter groups vs N. Amylin treatment normalized these altered trabecular structure parameters only in T2D rats (BV/TV: 29.4 ± 3.1 %, Tb.N: 1.54 ± 0.17 mm⁻¹ and Tb.Sp: 433 ± 54 μ m; all $p<0.05$ vs T2D-control); in addition, about a 30% decrease in both ES/BS and OcS/BS was also detected in T2D after amylin.

Conclusion: These data demonstrate, for the first time to our knowledge, that a sustained high physiological concentration of amylin exerts an osteogenic action in the femoral metaphysis in these T2D rats. Since this osteogenic effect of amylin is absent in our IR model, we hypothesize that this hormone might require an intact insulin signalling.

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Study on the effect of aldosterone excess on abnormal glucose metabolism

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Background and aims: Primary aldosteronism (PA) is often associated with glucose intolerance and diabetes. An impairment in glucose metabolism and insulin action may be induced by excess aldosterone, although its mechanism remains unresolved. Thus we intended to clarify the characteristics of glucose metabolism in PA.

Materials and methods: We enrolled 72 cases with PA (33 males and 39 females, mean age=52.7 years old) who had no history of diabetes. Localization of adrenal lesion was determined by adrenal venous sampling. The patients with Cushing or subclinical Cushing syndrome were excluded by 1mg dexamethasone suppression test. For the control group, we subjected 239 cases of essential hypertension (EHT) who were admitted to our hospital for general health check-up. EHT group was matched with PA group for age and BMI. 75g OGTT was performed to all the subjects, and plasma glucose and insulin were measured at 0, 30, 60, 90 and 120 minutes. The homeostatic model assessment (HOMA) was used to evaluate insulin sensitivity. We also compared Matsuda index (M-I), insulinogenic index (IGI) and disposition index (D-I) from the results of glucose and IRI response to OGTT. In addition, 26 patients went through OGTT before and one year after surgical treatment of PA.

Results: The frequency of abnormal glucose tolerance was not different between the PA and EHT group. However, HOMA-R was higher and M-I was lower in the PA patients with abnormal glucose tolerance compared with EHT patients, reflecting decreased insulin sensitivity in PA patients. The PA patients demonstrated higher acute insulin secretion determined as IGI than EHT patients. Moreover, when we compared the glucose metabolism of PA patients before and one year after adrenal resection, the glucose tolerance was apparently improved in 10 of 26 cases (38.5%) after surgical treatment.

Conclusion: These findings raise the possibility that aldosterone excess may cause impaired glucose metabolism in PA patients, and that abnormal glucose tolerance in PA is characterized by presence of decreased insulin sensitivity. Moreover, we should focus on the importance of aldosterone in pathogenesis of diabetes.

PS 032 Muscle insulin resistance

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Beneficial effect of fibroblast growth factor 21 to the insulin resistance induced by palmitate in skeletal muscle cells

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Background and aims: Insulin resistance is an important mechanism of type 2 diabetes mellitus. Fibroblast growth factor 21 (FGF21) has been identified as a potent metabolic regulator with specific effects not only on glucose and lipid metabolism, but also as a regulator of energy balance, especially in adipocytes and the liver. However, it is not yet clear whether FGF21 contributes to insulin resistance in skeletal muscle cells. Palmitate causes insulin resistance in skeletal muscle cells through the activation of a chronic inflammatory process. Here, we investigated the potential contribution of FGF21 to the insulin resistance induced by palmitate in skeletal muscle cells.

Materials and methods: First, confirming the contribution of FGF21 to the palmitate-induced insulin resistance, we determined 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NDBG) uptake and levels of proteins related to insulin signaling pathways (IRS-1, Akt) in human skeletal muscle myotubes (HSMM) exposed to palmitate (100, 200 μ M) for 24 h and compared those in HSMM exposed to both palmitate and different doses of recombinant FGF21 (100, 200 ng/mL). Second, to determine the mechanisms underlying the contribution of FGF21 to palmitate-induced insulin resistance, we compared levels of protein related to palmitate-induced insulin resistance (PKC- θ , IKK β , JNK, p38, I κ B α , NF- κ B) in HSMM exposed to palmitate and different doses of recombinant FGF21 (100, 200 ng/mL) for 24 h.

Results: Palmitate reduced the insulin-stimulated glucose uptake in HSMM. However, palmitate-reduced glucose uptake was restored by FGF21. Palmitate also inhibited phosphorylation of Akt, and thus impaired the insulin signaling pathway in HSMM, but FGF21 prevented the palmitate-inhibited phosphorylation of Akt. These results indicated that FGF21 prevented palmitate-induced insulin resistance in HSMM. Palmitate activated NF- κ B in HSMM, and thus impaired the action of insulin and initiated chronic inflammation in HSMM. However, FGF21 inhibited the palmitate-induced NF- κ B activation in HSMM. **Conclusion:** The results of the present study suggest that FGF21 prevents palmitate-induced insulin resistance in HSMM by inhibiting activation of stress kinase and NF- κ B.

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Functional characterisation of the Rab GTPase activating protein TBC1D1 *in vitro*

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Background and aims: TBC1D1 is a homolog of the insulin signal protein AS160 and regulates the insulin-stimulated glucose uptake in skeletal muscle. The biological activity of TBC1D1 is regulated by phosphorylation on multiple serine/threonine residues and strictly depends on its intrinsic Rab-GTPase activating protein (GAP) activity. A human variation (R125W) is associated with familial obesity, whereas a loss-of-function mutation of Tbc1d1 in lean SJL mice leads to increased fatty acid uptake and oxidation in skeletal muscle and thus protects from HFD-induced obesity and diabetes. The aim of this study is to investigate the molecular mechanism for regulating TBC1D1's GAP activity and to identify new Rab proteins within the TBC1D1 signal pathway. Therefore, we established an *in vitro* test procedure to functionally characterize the enzymatic specificity and Rab-GAP activity of purified recombinant TBC1D1.

Materials and methods: The mRNA expression level of 56 Rab-GTPases was quantified in skeletal muscle and white adipose tissue of lean and obese

mouse strains using Affimetrix Chip analysis. For establishment of an *in vitro* test procedure, cDNAs of 8 Rab-GTPases highly expressed in skeletal muscle, and the 48 kDa GAP domain of TBC1D1 as well as an inactive mutant (R941K), were cloned and expressed as glutathione-S-transferase (GST)-fusion proteins in *E. coli* and purified by glutathione affinity chromatography. In addition, full length histidine-tagged TBC1D1 (1255 aa) and the inactive mutant (His6-R941K) were produced by baculovirus-mediated expression in Sf9 insect cells. His6-TBC1D1 proteins were purified via Ni-NTA-affinity chromatography. The GAP activity of TBC1D1 was determined by measuring GTP turnover using isotope techniques.

Results: Soluble forms of the GST-Rab proteins and GST-GAP domains were purified from bacterial lysates with a yield of about 0.1 mg/l of culture. Of all Rab proteins tested, the recombinant TBC1D1-GAP domain showed highest specific activity to Rab10, which represents the most abundant Rab isoform in skeletal muscle. The GTPase activity of Rab10 was stimulated >10-fold in the presence of the GAP domain. The His6-tagged 160 kDa TBC1D1 protein was expressed in baculovirus-infected Sf9 cells and subsequently purified with high yield (0.3 mg/l of culture). First experiments showed catalytic GAP activity of His6-TBC1D1 towards GST-Rab10 *in vitro*. Nevertheless, purification and reaction conditions have to be optimised further to enhance the stability of His6-TBC1D1 and to improve the robustness of the test procedure.

Conclusion: The Rab10 isoform is a likely downstream target of TBC1D1 in the insulin signal cascade in skeletal muscle. Our assay now allows functional *in vitro* characterization of TBC1D1 and the search for TBC1D1 inhibitors as well as the development of novel pharmacological modulators of TBC1D1 that might be suitable for the treatment of obesity.

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Skeletal muscle mitochondrial cytochrome c oxidase activity is reduced in obese prediabetic individuals

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Background and aims: Obesity is considered a risk factor for the development of insulin resistance and type 2 diabetes. Evidence exists for the connection between the number and function of skeletal muscle mitochondria and insulin resistance. However, a causative role of mitochondrial dysfunction in obesity related type 2 diabetes development is unclear. Therefore, we compared skeletal muscle mitochondrial content and function in the group of young (31.2±1.1 yrs.) lean healthy men (n=18; 22.3±0.5 kg.m⁻²; body fat 16.8±1.0%) with that of obese/overweight men with normal glucose tolerance (n=14; 30.5±0.9 kg.m⁻²; body fat 28.9±1.3%), and with prediabetes (n=12; 30.2±0.8 kg.m⁻²; body fat 28.0±1.1%).

Materials and methods: Complex metabolic phenotyping included euglycemic hyperinsulinemic clamp (assessment of insulin sensitivity), oral glucose tolerance test, magnetic resonance imaging (adipose tissue size and distribution) and ¹H-MR spectroscopy (hepatic and intramyocellular fat content). Physical activity was monitored with accelerometers and “Beacke” activity questionnaire. Samples of vastus lateralis skeletal muscle were obtained by needle biopsy. The expression of mitochondrial markers cytochrome c, peroxisome proliferator activated receptor coactivator 1 alpha, ATP-Mg2+/Pi mitochondrial carrier SLC25A25 as well as mitochondrial DNA content were determined by qRT PCR. The activity of cytochrome c oxidase was measured by oximetry in permeabilised muscle fibers.

Results: Markers of mitochondrial biogenesis, activity (cytochrome c, ATP-Mg2+/Pi mitochondrial carrier) and mtDNA copy number did not differ between the groups. However, cytochrome c oxidase activity was decreased specifically in obese prediabetics. Activity of cytochrome c oxidase was negatively associated with % of body fat (p=0.031, r=0.383), subcutaneous (p=0.027, r=0.397) and visceral fat content (p=0.037, r=0.375), fat cell size (p=0.003, r=0.518), marginally with hepatic lipid content and positively with insulin sensitivity (p=0.027, r=0.394), sport index (p=0.015, r=0.420) and leisure-time index (p=0.034, r=0.371). Gene expression of the ATP-Mg2+/Pi mitochondrial carrier SLC25a25 which has been shown to decrease metabolic efficiency and physical endurance in mice, correlated negatively with several parameters of physical activity (number of steps/24h p=0.018, r=0.377, walking distance p=0.015, r=0.389, high intensity ambulatory activity/24h p=0.011, r=0.401) and with cytochrome c oxidase activity (p=0.026, r=0.387).

Conclusion: Our results indicate that reduced skeletal muscle mitochondrial function found in prediabetes, a clinical precursor of type 2 diabetes, might play an active role in the pathogenesis of type 2 diabetes. The skeletal muscle mitochondrial function mirrors metabolic phenotypes and the physical activity profile, pointing at the importance of physical activity in the maintenance of metabolic health.

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COX2 inhibition with celecoxib improves mitochondrial density in diabetic rat muscle

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Background and aims: Both mitochondrial dysfunction and alterations in mitochondrial DNA (mtDNA) are implications of type 2 diabetes mellitus and insulin resistance. Recent evidences suggest that metabolism of cyclooxygenase-2 (COX2) is associated with mitochondrial dysfunction and insulin resistance.

Materials and methods: We investigated the effect of celecoxib as a COX2 inhibitor on mtDNA density and insulin sensitivity using the obese diabetic OLETF rats and lean nondiabetic LETO rats. We produced severe insulin resistance in twenty-week-old male OLETF rats with high fat diet for 5 weeks. OLETF rats were further subdivided into two groups: control (in drinking water, n=10), celecoxib (15mg/kg, in drinking water, n=10).

Results: In high-fat diet OLETF rats, 5-week treatment with celecoxib did not change body weight (control vs celecoxib, 643±35 vs 653±63g), overnight-fasting glucose (150±62 vs 108±18mg/dL), and fasting insulin (1045±281 vs 895±221pmol/L). In OLETF rats, skeletal muscle mitochondria were severely destroyed and mtDNA density was decreased compare to lean LETO rats. These mitochondrial impairment was remarkably preserved in celecoxib-treatment OLETF rats (Figure).

Conclusion: COX2 inhibition with celecoxib in obese, insulin resistant diabetic rats might preserve mitochondrial dysfunction in skeletal muscle.

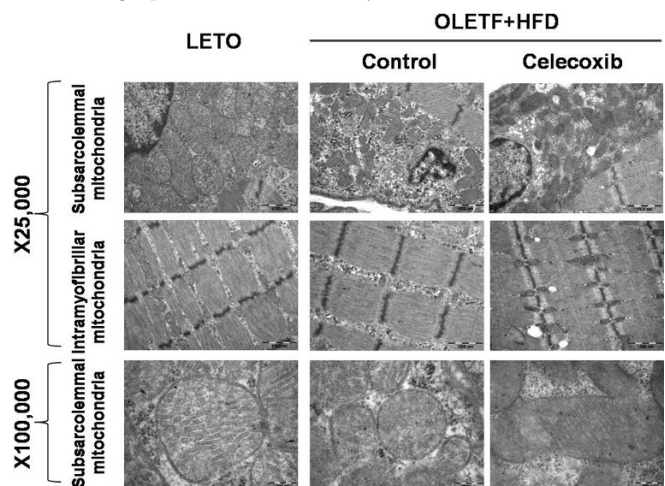


Figure. Electron microscopic findings of gastrocnemius muscle

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Leucine changes the timing and magnitude of insulin signalling in human myotube cultures, increasing baseline AKT, mTOR and ERK1/2 phosphorylation

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Background and aims: Phosphorylation events along the insulin signaling pathway are important in determining insulin sensitivity, which is altered in diabetes and obesity. There is an important interplay between amino acids

such as leucine and insulin signaling pathways which mediate insulin sensitivity and glucose disposal. This study's aim was to test the effect of leucine treatment on insulin signaling in a cell culture of differentiated human myotubes. Key target proteins along the insulin signaling pathway and their phosphorylation patterns at relevant sites were quantified in a time-dependent manner to elucidate the mechanisms by which leucine can induce insulin resistance in skeletal muscle, which has important implications for whole body glucose metabolism.

Materials and methods: Commercially available human myoblasts from skeletal muscle were induced to differentiate into myotubes by exposing them to DMEM:F12 with 2% horse serum and 50 µg/ml gentamycin for ten days, after which they were serum-starved overnight with 5.5 mmol/l glucose DMEM. Cells were then switched to a balanced salt solution for 1h and either treated with DMEM + 100 nmol/l insulin or primed with DMEM + 400 µmol/l leucine for 1h, followed by treatment with DMEM + 100 nmol/l insulin + 400 µmol/l leucine at five time points, after which cells were lysed and total and phosphorylated protein targets were quantified by Western blot and/or Nanopro immunoassay. Medium glucose concentrations for each time point were enzymatically determined.

Results: Leucine priming increased baseline AKT phosphorylation at Ser473 more than fourfold relative to without leucine priming ($p<0.04$, two-tailed t test). In the absence of leucine, insulin induced maximal AKT phosphorylation at Ser473 at 10 min (tenfold relative to baseline). In the presence of leucine, insulin induced maximal Ser473 AKT phosphorylation earlier, at 5 min, but the magnitude was reduced (twofold relative to baseline), and the duration was reduced, returning back to pre-insulin levels by 10 min. In the absence of leucine, phosphorylation was sustained fivefold relative to baseline even after 60 min insulin exposure. Absolute magnitude of peak AKT Ser473 phosphorylation was increased 41% with insulin in the absence of leucine relative to in the presence of leucine. Nanopro immunoassay results confirmed Western blot findings for the effect of leucine on AKT phosphorylation. Leucine priming increased baseline ppERK12/ERK12 (Thr202/Tyr204) more than twofold ($p<0.03$, two-tailed t test) and p-mTOR/mTOR (Ser2448) twofold ($p<0.006$, two-tailed t test) relative to without leucine priming. In addition, the presence of leucine results in a more rapid but smaller acute drop in medium glucose concentrations (5 min, Δ glucose = -0.82 mmol/l) relative to insulin alone (10 min, Δ glucose = -1.79 mmol/l) though the effect of leucine on medium glucose is no longer apparent after 60 min.

Conclusion: Leucine attenuates the insulin-induced decline in glucose levels in the culture media by blunting insulin signaling, increasing baseline phosphorylation on AKT, ERK12 and mTOR, and reducing insulin's induction of phosphorylation events.

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Relationships between mitochondrial function and metabolic flexibility in type 2 diabetes mellitus

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Background and aims: Mitochondrial dysfunction, metabolic inflexibility and intramuscular fat accumulation (IMCL) have been suggested to underlie the development of insulin resistance, but their interrelationship has not been well established. Here we determined these aspects in a relatively large group of type 2 diabetic patients and control subjects and determined the relationship with the oxidative and non-oxidative aspects of insulin resistance.

Materials and methods: In total 49 non-insulin dependent diabetic patients and 54 control subjects were enrolled, in which a 3-h hyperinsulinemic-euglycemic (40 mU /m²/min) clamp was performed, primed with an infusion of [6,6-2H2]glucose (0.04 mg/kg/min) to determine rates of glucose appearance (Ra) and disposal (Rd). Indirect calorimetry was performed to assess substrate metabolism. A muscle biopsy was taken, in which IMCL was determined with oil red O staining. In 31 control subjects and 30 diabetic patients PCr-recovery was measured for determination of *in vivo* mitochondrial function. Differences were analyzed with a one-way ANOVA. Linear regression was performed after adjustment for age, BMI and percentage of body fat. Data are reported as means \pm SE.

Results: Type 2 diabetic patients and control subjects had similar body weights (91.7 \pm 11.9 vs. 92.4 \pm 11.3 kg), BMI (29.8 \pm 3.1 vs. 29.4 \pm 3.3 kg/m²) and age (61.2 \pm 3.7 vs. 59.8 \pm 4.8 years). IMCL was not significantly different between the groups (0.96 \pm 0.66 vs. 1.10 \pm 0.64 AU). Baseline Rd was significantly higher in diabetic patients compared with control subjects (11.9 \pm 3.9 vs. 9.4 \pm 2.3

µmol/kg/min, $p<0.01$), with a higher glucose oxidation (8.3 \pm 2.6 vs. 6.7 \pm 2.2 µmol/kg/min, $p<0.05$), and no differences in non-oxidative glucose disposal (NOGD). Rd during insulin stimulation was significantly lower in diabetic patients (20.3 \pm 7.5 vs. 28.7 \pm 9.5 µmol/kg/min, $p<0.01$). This was due to a lower NOGD (8.4 \pm 6.9 vs. 15.4 \pm 8.1 µmol/kg/min $p<0.01$) and a lower glucose oxidation rate (12.0 \pm 3.3 vs. 13.7 \pm 3.7 µmol/kg/min, $p<0.05$) in diabetic patients upon insulin stimulation. The respiratory quotient (RER) was significantly higher at baseline (0.82 \pm 0.03 vs. 0.79 \pm 0.03, $p<0.01$) and was lower upon insulin stimulation (0.87 \pm 0.04 vs. 0.89 \pm 0.04, $p<0.05$) in diabetic patients. This resulted in a lower metabolic flexibility in diabetic patients (+0.05 \pm 0.03 vs. +0.09 \pm 0.05, $p<0.01$). Diabetic patients had a prolonged PCr-recovery half-time (PCr- $t_{1/2}$) by 12.5% compared to control subjects (22.3 \pm 6.9 vs. 19.8 \pm 4.5 s, respectively; $p<0.01$). PCr-recovery rates did not correlate with IMCL, but there was a negative correlation with insulin stimulated Rd ($R^2=0.10$, $p<0.05$). Metabolic flexibility not only correlated with Rd, but also independently correlated with *in vivo* mitochondrial function (Rd $R^2=0.17$, $p<0.01$, PCr- $t_{1/2}$ $R^2=0.07$, $p<0.05$).

Conclusion: These results indicate that defects in mitochondrial function, irrespective of lipid accumulation, might contribute to defects in insulin stimulated glucose disposal and oxidation in type 2 diabetes.

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Endoplasmic reticulum stress does not mediate palmitate-induced insulin resistance in muscle cells

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Background and aims: Insulin resistance observed in skeletal muscle is a main feature of obesity and type 2 diabetes. Both diseases are interconnected, and elevated plasma concentrations of non-esterified fatty acids (NEFA) are always observed, leading to lipotoxicity through the over-accumulation of lipid derivatives such as ceramide in non-adipose tissues. We, and others, have shown that palmitate-generated ceramide inhibited insulin signalling in muscle cells by targeting specifically the protein kinase B, or Akt (PKB/Akt), a kinase implicated in the stimulation of glycogen synthesis and glucose transport. Recent experiments in liver and adipocyte cell lines indicate that palmitate could also initiate a response called Endoplasmic reticulum (ER) stress. In liver, ER stress has been shown to play a role in the pathogenesis of diabetes, contributing to steatosis, β -cell loss and insulin resistance through the activation of stress-activating kinases such as the Jun Kinase (JNK). Because ceramide biosynthesis from palmitate occurred at the level of the ER, our hypothesis was that the deleterious action of palmitate on the insulin signalling pathway could also involve an ER stress in muscle cells.

Materials and methods: We used C2C12 myotubes that were treated either with 0.75mM palmitate or 0.5µg/ml tunicamycin for 16h. Then total lysates and RNA were prepared for western blotting or quantitative RT-PCR respectively.

Results: Incubation of C2C12 myotubes for 16h with palmitate or tunicamycin, an agent that is known to induce hepatic insulin resistance through the activation of ER stress, induced an inhibition of insulin-stimulated PKB/Akt. In parallel, an increase in ER stress markers was observed in response to both palmitate and tunicamycin in these cells (i.e. phosphorylation of eIF2 α , splicing of XBP-1, activation of ER stress chaperones and foldases). We then examined whether tauroursodeoxycholic acid (TUDCA), a well-established chemical chaperone that has been demonstrated to inhibit ER stress, could improve insulin resistance in both cases. Results showed that pre-incubation of cells with TUDCA only prevented tunicamycin-induced insulin resistance, with no effect on the harmful action of palmitate on PKB/Akt. Our hypothesis to explain this discrepancy was that the level of activation of the ER stress induced by the lipid may be not high enough. Indeed, tunicamycin induced robust activation of the IRE1/JNK pathway, leading to serine phosphorylation-induced inhibition of IRS1. On the other side, palmitate only induced a mild activation of the IRE1/JNK pathway, with no phosphorylation of IRS1 on serine residues.

Conclusion: These data show that the onset of insulin resistance induced by palmitate is not related to ER stress in muscle cells. Production of ceramide from palmitate remains a crucial step to downregulate the insulin signalling pathway at the level of PKB/Akt. However we cannot exclude that a chronic exposure of the muscle cells to saturated fatty acids, like it could be the case in obesity, may induce a more important ER stress than seen in our conditions, therefore affecting in that case the insulin pathway.

Supported by: INSERM

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Strongly increased intramyocellular lipids, but normal muscular mitochondrial oxidative capacity in adipose triglyceride lipase deficient mice

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Background and aims: Adipose triglyceride lipase (ATGL) is the rate-limiting enzyme of triglyceride hydrolysis and is mainly expressed in adipocytes and myocytes, thus ATGL-KO mice show abnormal triglyceride accumulation in those tissues. Intramyocellular lipids (IMCL) are often used as biomarker of insulin resistance and type 2 diabetes, potentially reflecting lipotoxic effects on mitochondrial function. Whether IMCL accumulation per se has adverse effects on mitochondria is unclear. Therefore, we assessed IMCL levels and mitochondrial oxidative capacity of ATGL-KO mice *in vivo* with ¹HMRS and gated dynamic ³¹PMRS of contracting calf-muscles.

Materials and methods: Adult ATGL KO (n=9) and WT (n=10) mice were included in these experiments. ipGTT was done in all mice after 6h of fasting with a glucose bolus of 1.5g/kg. Plasma glucose levels were analyzed with a glucometer at 0, 15, 30, 60, 90 and 120min post-load. *In vivo* MR spectroscopy was performed at 7T (Clinscan, Bruker Biospin). The IMCL assessment was done in tibialis anterior using single voxel ¹HMRS. Briefly, all animals were anaesthetized with isoflurane and their left hindleg positioned under a ¹H surface coil and oriented with the magnetic field. IMCL level was quantified from the ¹H spectra and normalized to [creatine]. To investigate whether mitochondrial oxidative capacity was affected by the IMCL accumulation in ATGL-KO mice, we assessed phosphocreatine (PCr) recovery time in post-contracted muscles. Tetanic contraction of the calf muscle was achieved by stimulation of the sciatic nerve and occurred every 3 seconds over one minute. Eight time series of ³¹PMRS spectra were acquired before, during and after the muscle contraction. The PCr recovery curve was fitted with a mono-exponential function and recovery time calculated. Samples from the exercised and non-exercised calf muscles were freeze-clamped and extracted to determine glycogen level using the hot-alkali method.

Results: The ¹HMRS examination of skeletal muscle showed that IMCL/Cr ratio of ATGL-KO was ~5 fold higher than their counterparts (5.6±1.1 vs 1.1±0.3, p=0.0007). IpGTT revealed that ATGL-KO mice were more glucose tolerant than the WT mice with an AUC of 1067±54 vs 1277±56, p=0.01, respectively. ³¹P spectra showed that electro-stimulated muscles of ATGL-KO and WT had similar PCr depletions (~40–50%) and analysis of PCr recovery curves resulted in similar recovery times for ATGL-KO (59.8±6.6s) and WT (57.2±6.3s). Glycogen levels in ATGL-KO muscle were lower than in WT, in contracted (7.1±1.9 vs 10.7±1.7 μmolglucosyl/gww) and non-contracted muscle (15.8±1.7 vs 25.1±2.3 μmolglucosyl/gww, p<0.01).

Conclusion: Skeletal muscle of ATGL-KO mice have a ~5-fold increase in IMCL pool, but this did not affect mitochondrial oxidative capacity. The hampered fatty acid catabolism boosts glucose oxidation as shown by improved glucose tolerance and decreased glycogen stores.

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Electric pulse stimulation of human skeletal muscle cells induces enhanced insulin response and anti-inflammatory signalling

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Background and aims: In the context of obesity, physical inactivity is associated with chronic systemic inflammation and represents a major risk factor for the pathogenesis of type 2 diabetes. Several studies could show that regular activity considerably improves insulin sensitivity and skeletal muscle metabolism. For our recently established contraction model we used *in vitro* electric pulse stimulation (EPS) of primary human skeletal muscle (hSkM) cells. Cells start to contract after few hours, AMPK is activated and secretion of myokines like IL-6 and VEGF were increased as it was shown before for exercise of skeletal muscle *in vivo*. Adipocyte conditioned medium (CM)-induced impairment of insulin signaling was completely prevented by contractile activity of hSkM cells. Therefore, the aim of this study was to further

characterize beneficial effects of muscle activity and to identify underlying signaling pathways and mechanisms.

Materials and methods: Differentiated hSkM cells were stimulated with a C-pulse generator for up to 24 h (1 Hz, 2 ms, 11.5 V). Cells were stimulated with 100 nM insulin for 30 min and 2-DOG glucose uptake was determined. For analysis of inflammatory signaling hSkM cells were incubated with 50 pg/ml TNFα for 5, 10 and 20 min, with 1 ng/ml MCP-1, 2 μg/ml chemerin and CM for 30 min. Protein expression and phosphorylation of Akt, GSK3α/β and NFκB cascade were analyzed by Western blotting.

Results: Glucose uptake was significantly increased after insulin stimulation (2.5 fold) and by EPS alone (2.2 fold). Cells showed a substantial increase in insulin-stimulated glucose uptake after EPS compared to basal control (4.6 fold) and synergistically to insulin-stimulated control (1.8 fold, n>3). MCP-1 and chemerin induced impairment of insulin signaling by 32 and 39 % at the level of Akt phosphorylation (n=4). This effect was completely prevented by contractile activity of hSkM cells. CM and chemerin induced an activation of NFκB (1.8 fold and 1.5 fold, n=4), while MCP-1 induced the activation of p44/p42 MAPK (1.5 fold). Both effects on NFκB and p44/p42 MAPK activation were diminished to control level after EPS. Incubation of hSkM cells with TNFα induced pro-inflammatory signaling reaching a maximal activation of NFκB after 10 min (3.2 fold, n>4). The application of EPS during the treatment with TNFα diminished the activation of NFκB at all analyzed time points compared to unstimulated control (1.7 fold after 10min, n>4). NFκB and IKKβ protein level were significantly reduced by 35 % after 8 h EPS compared to unstimulated controls (n=4), while protein level of IKKα and IκBα were not changed. Incubation with TNFα diminished IκBα protein level significantly to 36 % after 20 min, while TNFα had no effect on IκBα protein level of contracting cells (n=4).

Conclusion: Our results provide direct evidence that muscle contractile activity triggered by EPS synergistically improves insulin response as demonstrated by an increased glucose uptake. Furthermore, EPS could prevent MCP-1 and chemerin-induced impairment of insulin signaling. This positive effect can in part be explained by the anti-inflammatory effect of contractile activity, which was demonstrated by a diminished activation of key proteins in the pro-inflammatory NFκB pathway by adipokines. In summary, our model provides a unique tool which shows that the anti-inflammatory potential of contraction might mediate the beneficial effect of muscle contraction.

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Improved intramyocellular triglyceride fatty acid content and composition during moderate weight loss: impact of sex

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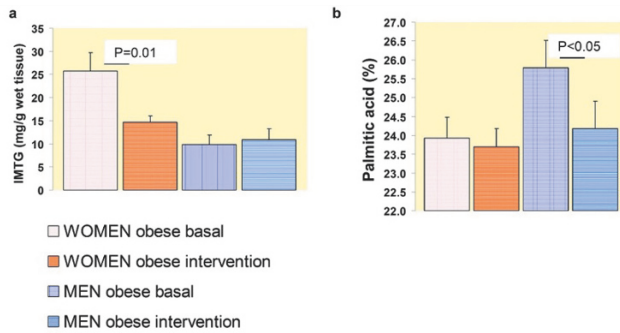
Background and aims: The content and fatty acid composition of intramyocellular triglyceride (IMTG) implicates on glucose metabolism and may show gender specificity. We investigated whether a low-calorie dietary intervention influences IMTG content and composition with emphasis on sex differences.

Materials and methods: Twenty-one insulin resistant obese subjects (12/9 women/men of whom 4/3 had diabetes) of mean BMI=36.5 kg/m² completed a 6-months low-calorie dietary intervention (approx. 7 MJ/d). Dietary advising emphasized low intake of fish-oil by recommending lean fish and prohibiting fatty fish and fish-oil supplements. IMTG content and fatty acid composition was determined in vastus lateralis biopsies by thin-layer and gas-liquid chromatography.

Results: Obese women lost 43% of IMTG (from 25.8 mg/g wet weight, P=0.01), whereas in obese men IMTG remained unchanged at a lower level (baseline 9.8 mg/g wet weight, P=ns). Long-chain-polyunsaturated fatty acids (LCPUFA) and LCPUFA-n-3 of IMTG increased both in women and men (84%, P<0.001 and 107%, P<0.001). Interestingly, the content of the potential diabetogenic palmitic acid (C16:0) decreased in men only (-6.2%, P<0.05) rendering gender difference significant (P<0.05). Subjects experienced weight-loss (5.1 kg, P<0.001), less stress on betacell (decrease in fasting C-peptide of 15%, P<0.05), decreased fasting glucose (9% from 7.1 mmol/L, P=0.057), and decreased glycosylated hemoglobin (0.5 percentage point from 6.7%, P=0.081), respectively, with no significant gender difference observed.

Conclusion: Moderate weight-loss in obese subjects was associated with decreased IMTG content in women only, increased LCPUFA-n-3 irrespective of gender, and reduced palmitic acid content in men. Gender specific responses

of intramyocellular lipids to diet induced weight-loss may be linked mechanistically to improvement of glucose metabolism.



Effect of moderate weight loss on intramyocellular triglyceride (IMTG) content (panel a) and diabetogenic IMTG palmitic acid (panel b) in obese insulin resistant women (n=12) and men (n=9)

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PS 033 Glucose transport

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GLUT2 and its transcriptional regulation by HNF-4α: participation in kidney and liver

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Background and aims: In diabetes mellitus, increased expression of GLUT2, as well as, augmented production of extracellular matrix proteins by mesangial cells could collaborate to development of glomerulosclerosis. Moreover, the increased expression of GLUT2 may be associated with NASH (Nonalcoholic Steatohepatitis) and Fanconi Bickel syndrome. We have investigated the effect of insulin and demonstrated a downregulated GLUT2 expression in kidney and liver, which returned to non diabetic levels, and points out the glucose concentration as the key regulator of this gene. These results indicate a transcriptional control was involved, and HNF-4α was identified and characterized in GLUT2 gene promoter. The aim of present study was to investigate the role of HNF-4α in kidney and liver of diabetic rats and insulin treated diabetic rats.

Materials and methods: We studied 3 month old Wistar rats, randomly allocated into non diabetic (C), diabetic (D), diabetic treated with saline (DS) and insulin (I-I, 4 and 6 days of treatment). The experimental protocols were submitted to the Ethics Committee for Experimentation with Laboratory Animals of the ICB-USP (protocol 41/2009). Diabetes was induced by single endovenous dose of alloxan (38 mg/Kg). The mRNA of HNF-4α was analyzed by RT-PCR. The HNF-4α binding activity of nuclear protein into GLUT2 promoter was analyzed by Electrophoretic Mobility Assay.

Results: In comparison to C animals, D rats showed: hyperglycemia, polyuria, glycosuria without ketonuria, increased GLUT2 mRNA content in kidney (P<0,001) and liver (P<0,0001), increased HNF-4α mRNA content (P<0,01) and binding activity of nuclear protein into GLUT2 promoter (P<0,0001) in kidney and increased HNF-4α mRNA content (P<0,01) and binding activity of nuclear protein into GLUT2 promoter (P<0,01) in liver. In comparison to D animals, the DI animals showed: a) reduction of glycemia in DI rats 10% (1 day), 22% (4 days) and 48% (6 days); b) progressive increase of body weight 2% (1 day), 5,4% (4 days) and 24% (6 days); c) decrease of GLUT2 mRNA content after 1 day of insulin treatment (P<0,05) in kidney and after 4 days of insulin treatment in liver, e) decrease of HNF-4α mRNA content after 6 days of insulin treatment (P<0,05) in kidney and liver and decreased of the HNF-4α binding activity of nuclear protein into GLUT2 promoter, after 6 days of insulin treatment (P<0,001) in kidney and liver.

Conclusion: The study showed that HNF-4α plays important role in the GLUT2 gene regulation, which was demonstrated by increased HNF-4α gene expression and binding activity into GLUT2 promoter in diabetic rats. These data suggest that in insulin treatment HNF-4α also could be an important transcriptional regulator of GLUT2 gene and probably acts as mediator on GLUT2 gene in kidney and liver of insulin treated diabetic rats.

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Determining the renal threshold for glucose excretion: validation of a new method

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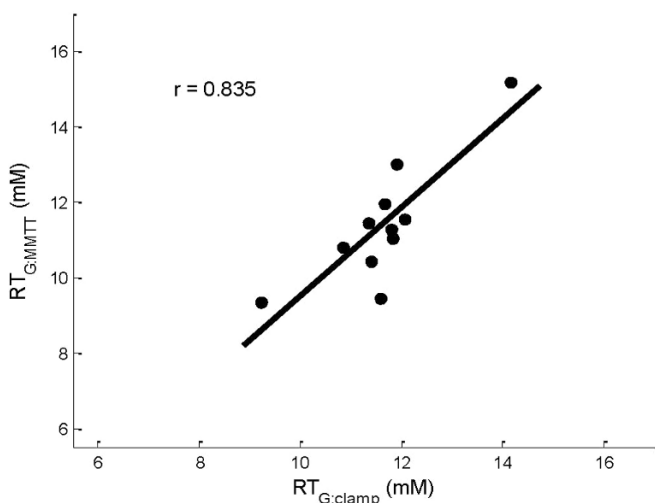
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Background and aims: There is a threshold relationship between urinary glucose excretion (UGE) and blood glucose (BG) in which there is virtually no UGE under euglycemic conditions and UGE increases with BG when BG exceeds the renal threshold for glucose (RT_G). Recent reports of increased renal glucose transporter expression in subjects with type 2 diabetes mellitus (T2DM) and the emergence of sodium glucose co-transporters 2 (SGLT2) inhibitors as potential treatments for T2DM have led to increased interest in renal glucose handling. However, the only accepted methods to determine RT_G require multi-step hyperglycemic clamps or graded glucose infusions with frequent blood and urine sampling and are therefore limited to small studies. The purpose of this study is to validate a new method for determining RT_G that is suitable for use in larger studies.

Materials and methods: Fourteen subjects with T2DM (mean age = 56 y, BMI = 30 kg/m², HbA1c = 8.3%, eGFR = 89 mL/min/1.73 m²) who were either

on a stable metformin regimen or no antihyperglycemic agents were studied. On day 1, a standard mixed meal tolerance test (MMTT) was performed and BG profiles and UGE were measured over 4 hours. RT_{GMMTT} was determined using an extension of the method used to determine $RT_{GMMTT}^{phosphate}$ that accounts for intraday BG variability. The UGE vs BG relationship was approximated by a perfect threshold relationship: rate of UGE = $GFR \times (BG - RTG)$ if $BG > RTG$ and rate of UGE = 0 if $BG \leq RTG$. Using measured BG and measured creatinine clearance to estimate GFR, this equation was integrated over the 0–4 hour interval of the MMTT, giving the calculated UGE amount as a function of RT_G . The unique value of RT_G making calculated UGE = measured UGE was determined using an iterative procedure. On day 2, the subjects underwent a 5-step hyperglycemic clamp ($BG = 7, 9.5, 12, 14.5, 17$ mM), and UGE and BG were measured during the last 1.5 hours of each step. RT_{Gclamp} was determined using nonlinear regression of the measured BG and UGE rates during the 5 steps. Application of either procedure requires BG exceeding RT_G during the experiment so that an appreciable amount of UGE is measured.

Results: RT_{Gclamp} was determined in all 14 subjects, with mean \pm SD $RT_{Gclamp} = 12.0 \pm 1.3$ mM. In 3 subjects, BG during the MMTT remained well below their RT_{Gclamp} ; these subjects had very little UGE during the MMTT (as expected) and their RT_{GMMTT} could not be determined. In the 11 subjects in whom RT_G was determined using both methods, mean values were similar for both methods ($RT_{GMMTT} = 11.4 \pm 1.6$ mM and $RT_{Gclamp} = 11.6 \pm 1.2$ mM) and the values obtained by the 2 methods were highly correlated (Figure), demonstrating good agreement between the 2 methods for determining RT_G . **Conclusion:** Using the methodology developed, RT_G may be reliably estimated in subjects with T2DM using UGE and BG measurements taken during an MMTT and estimated GFR values. The new MMTT-based method is well suited for determining RT_G in larger studies.



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Key role of BRS-3 receptor in the glucose metabolism in human skeletal muscle

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Background and aims: It has been previously informed, an important role of human BRS-3 (hBRS-3) receptor in glucose homeostasis; its agonist peptide -D-Tyr⁶-β-Ala¹¹, Phe¹³, Nle¹⁴]bombesin₆₋₁₄ (BRS-3-AP)- induced, in normal human skeletal muscle, a clear stimulation of glucose transport (GT) which was abolished by wortmanin -PI3K/PKB inhibitor- and PD98059 -MAPKS inhibitor-, as well as increased phosphorylation of PKB and MAPKS; it was also shown that hBRS-3 gene expression was lower in diabetic and/or obese patients compared to that in normal. Furthermore, gene deletion of BRS-3 in mice leads to obesity, associated with metabolic disorders. The aim of this

work was to widen the knowledge of BRS-3 signalling pathway, using the BRS-3-AP, and to explore its effect on glucose transport and metabolism in normal human muscle.

Materials and methods: Myocytes were prepared from skeletal muscle pieces, obtained previous informed consent given, from 19 subjects (10F/9M; age: 48±2 yrs; fasting plasma glucose: 96±3 mg/dl; cholesterol: 188±12; tryglycerides: 104±12), with normal carbohydrate metabolism, undergoing surgery. We measured: PI3K -PIP₃ formation-, p70s6k phosphorylation -Western blot-, glut-4 gene expression -real time-PCR-, GT -³H-2-deoxy-D-glucose incorporation-, in the absence and presence of BRS-3-AP (10⁻⁸ M) and without (control) and with 10⁻⁷ M rapamycin -p70s6K inhibitor-; glycogen synthase a (GSa) and glycogen synthesis (GS) by using radioactive glucose precursors; insulin was included as positive control.

Results: BRS-3-AP at 10⁻⁹ M induced an increase in PI3K activity (154±10% control, p<0.05), while failing to modify p70s6k phosphorylation (10⁻¹⁰ M: 100±2% control); however, at 10⁻⁹ and 10⁻⁸ M, a stimulation (123±10% control and 176±22%, p<0.02 or lower, respectively) was detected in the activity of the enzyme similar to that by 10⁻⁸ M insulin (182±17%, p<0.001). BRS-3-AP, at 10⁻⁸ M increased (p<0.05) the level of glut-4 gene expression (7.540 ± 0.067 times, up-regulated, respect control: 1.00 ± 0.01). Maximal glucose uptake was detected by 10⁻⁸ M BRS-3-AP (175±7% control, p<0.001) which was not affected by the additional presence of rapamycin (161±7%). BRS-3-AP caused a concentration-related stimulation in GSa activity, which was already significant at 10⁻¹⁰ M (149±7% control, p<0.05) higher at 10⁻⁹ M (162±7%, p<0.05), and maximal at 10⁻⁸ M (207±18%, p<0.01), effect which was similar to that induced by 10⁻⁹ M insulin (183±19% control, p<0.05). GS was significantly (p<0.05) stimulated by either 10⁻⁹ M BRS-3-AP or insulin (168±23% control and 165±13, respectively) and maximal at 10⁻⁸ M BRS-3-AP (257±23%).

Conclusion: These results confirm the key role of human BRS-3 in the glucose metabolism, and add support to the idea of using this receptor as a molecular target, and/or its agonist peptide, as a tool in the therapy of obesity and diabetes.

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Receptor subtype-3 (BRS-3): a candidate as therapeutic molecular target in obesity

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Background and aims: BRS-3 deficient mice develop a mild-late-onset obesity with metabolic disorders. Human skeletal muscle expresses functional BRS-3, and its gene expression is lower in diabetic and/or obese patients compared to normal. Moreover, we reported that in myocytes from normal subjects, the synthetic agonist peptide BRS-3 [D-Tyr⁶-β-Ala¹¹, Phe¹³, Nle¹⁴] bombesin₆₋₁₄ (BRS-3-AP) induces a significant increase in glut-4, glucose transport (GT), glycogen synthase a activity (GSa), glycogen synthesis (GS), and phosphorylation of protein kinases. The aim of this study was to gain insight into the BRS-3 signalling pathways in obese human myocytes, by using the BRS-3-AP as a ligand, and to investigate its effect on glut-4 expression, GT, GSa and GS.

Materials and methods: Myocytes were established from pieces of skeletal muscle obtained, previous informed consent given, from 14 obese patients (1F/13M; age: 44±3 yrs; fasting plasma glucose: 115±6 mg/dl; cholesterol: 167±14; tryglycerides: 170±23; BMI: 45±2 kg/m²), undergoing surgery. We measured p42/44 MAPKS activity -Western blot-, glut-4 gene expression -real time-PCR-, GT -³H-2-deoxy-D-glucose-, GSa -UDP-[¹⁴C]glucose- and GS -D-[U-¹⁴C]glucose-, in the absence (control) and presence of BRS-3-AP; insulin was included in all experiments as positive control.

Results: At 10⁻⁹ M, BRS-3-AP induced an increase (p<0.01 or lower) in the phosphorylation level of p42/44 MAPKS (p42: 197±26% control; p44: 166±24%), even higher than that by equimolar concentrations of insulin (p42: 141±6% control; p44: 136±8%) or the synthetic ligand in cells from normal subject. BRS-3-AP, at 10⁻¹⁰ M increased (p<0.05) the level of glut-4 gene expression (2.000 ± 0.023 times, up-regulated, respect control: 1.000 ± 0.006); also, it caused a concentration-related stimulation of GT, which was detectable at 10⁻¹⁰ M (163±10% control, (p<0.01), maximal at 10⁻⁹ M (226±14%,

$p < 0.001$), and maintained thereafter up to 10^{-7} M (10^{-8} M: $198 \pm 14\%$, $p < 0.001$; 10^{-7} M: $201 \pm 21\%$, $p < 0.001$), showing a higher potency ($p < 0.01$) in obese than in normal myocytes (10^{-9} M: $176 \pm 11\%$). In addition, the BRS-3 synthetic agonist significantly ($p < 0.05$) increased of GSa (10^{-9} M: $159 \pm 26\%$ control) and GS (10^{-9} M: $159 \pm 10\%$; 10^{-8} M $167 \pm 8\%$).

Conclusion: In myocytes from obese patients, BRS-3-AP not only increases protein kinase activities, improves GT and glucose metabolism but also the cells seem to be more sensitive to this analogue than normal myocytes. These results point out that BRS-3 receptor could be used as a molecular target, and/or its agonist peptide, in the therapy of obesity.

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Effect of methylmercury on insulin-stimulated glucose uptake in mouse skeletal muscle

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Background and aims: There is increasing evidence suggesting a role for environmental contaminants in the epidemic of the metabolic syndrome. For instance, several studies reported an association between increased body burdens of persistent organic pollutants (POPs) and type 2 diabetes as well as cardiovascular complications. Recently, we demonstrated that POPs commonly present in our food chains contribute to insulin resistance and impair glucose and lipid homeostasis in animals. The aim of the present study was to investigate whether methylmercury (MeHg), a toxic metal that accumulates in fish and sea mammals, could impair insulin action in mouse skeletal muscles.

Materials and methods: Male C57BL/6J mice (9 weeks of age) fed standard control diet were fasted for 5 h and anaesthetized by an intraperitoneal injection of pentobarbital (50 mg/ml). Soleus muscles (slow-twitch fiber) were dissected out, and insulin-stimulated glucose uptake and insulin signalling were assessed. For glucose uptake investigations, muscles were pre-incubated for at least 40 min in Krebs Henseleit buffer containing 5.5 mM glucose, 2 mM pyruvate, 5 mM HEPES and 0.1 % BSA, pH 7.4, and a continuous flow of gas (95 % O₂ - 5 % CO₂) was maintained during the incubation. After pre-incubation, muscles were incubated with 0, 1, 3, 10, 20, 50, 75, or 100 nM MeHg for 60 min. Other muscles were incubated with MeHg (75 nM) and N-acetyl-L-cysteine (NAC; 75 nM) or Selenium (Se; 75 nM). Then, muscles were incubated for 30 min with tracer amount of 2-[³H] deoxy-D-glucose and [¹⁴C]-mannitol in the presence or absence of insulin (60 and 0 nM, respectively). For insulin signalling investigations, western blots were performed to analyse total Akt and Akt phosphorylation (Ser⁴⁷³).

Results: Basal muscle glucose uptake did not change in the absence or presence of MeHg (75 nM). In muscles non-exposed to MeHg, insulin increased glucose uptake by 3-fold compared with basal glucose uptake. Incubation with low MeHg concentrations (1, 3, 10 nM) did not affect insulin-stimulated glucose uptake. At higher concentrations, MeHg dose-dependently inhibited insulin-stimulated glucose uptake (20 nM MeHg: - 31.4%, 50 nM MeHg: -32.5%, 75 nM MeHg: -51.4%, 100 nM MeHg: -65.9%). Consistent with these findings, we found that MeHg reduced insulin-stimulated phosphorylation of Akt, a key insulin signalling protein for glucose uptake. There was no effect of MeHg on total Akt expression. To investigate whether antioxidant could prevent the inhibitory effect of MeHg on insulin-stimulated glucose uptake, muscles were incubated with NAC or Se. NAC was able to totally prevent the inhibitory effect of MeHg on insulin-stimulated glucose uptake, while inclusion of Se showed no protective effect.

Conclusion: These results indicate that MeHg can interact with insulin action and impair glucose uptake in skeletal muscles. The antioxidant NAC but not Se can prevent the negative effect of MeHg on glucose uptake.

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Methylglyoxal stimulates GLUT4-driven glucose-uptake and induces oxidative stress

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Background and aims: Methylglyoxal (MG), a highly reactive α -dicarbonyl metabolite of glucose degradation pathways, protein and fatty acid metabo-

lism, plays an important role in the pathogenesis of diabetic complications. In diabetes, hyperglycemia triggers enhanced production of MG and increased generation of advanced glycation endproducts (AGEs). In a non-enzymatic reaction, MG reacts with arginine residues of proteins to form the AGEs argpyrimidine and hydroimidazolone. Glyoxalase 1 (GLO1), together with glyoxalase 2 and the co-factor glutathione, constitute the glyoxalase system, which is responsible for the detoxification of MG. A GLO1 specific knock down results in accumulation of MG in targeted cells. In this study the effects of MG-stimulation and down regulation of its detoxification system on insulin signaling and translocation of GLUT4 in myoblasts were investigated. **Methods:** L6 myoblasts cells, stably expressing c-myc epitope-tagged GLUT4, were transfected with GLO1-specific siRNA for 72h and cultured with 25mM glucose medium or stimulated with MG for 24h cultured with 5 mM glucose medium. Then cells were serum starved 4h before insulin stimulation for 1h. The impacts of the GLO1 knock down and MG treatment on the expression and phosphorylation of insulin-receptor substrate-1 (IRS-1) and AKT was analyzed by immunoblotting. The effects of the knock down and the MG treatment on the translocation of GLUT4, the glucose uptake and on oxidative stress were investigated by flow cytometry.

Results: Both, MG treatment as well as GLO1 knock down resulted in increased translocation of GLUT4 to the plasma membrane without insulin stimulation (100 μ M MG: 122% ($p > 0.05$), 400 μ M MG: 211% ($p < 0.001$) vs. 0 μ M MG and GLO1 knock down vs. scrambled siRNA: 239% ($p < 0.01$)). Enhanced glucose uptake was detected. Down regulation of GLO1 as well as MG treatment resulted in decreased expression of AKT but in increased phosphorylation of AKT: ratio phosphoAKT to totalAKT 100 μ M MG 350% ($p > 0.05$); 400 μ M MG 525% ($p < 0.01$) vs. 0 μ M MG, GLO1 knock down vs. scrambled siRNA: 243% ($p < 0.01$), respectively. The GLO1 knock down as well as MG treatment have no significant effect on the expression and phosphorylation of IRS-1. In addition, measurement of reactive oxygen species (ROS) showed that treatment with MG and down regulation of GLO1 induced oxidative stress.

Conclusion: MG, either intracellularly accumulated via GLO1 knockdown or extracellularly added, induces glucose uptake via an enhancement of GLUT4 translocation and oxidative stress. The interplay between these two phenomena is under current research using this cell line.

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Effect of chronic caffeine administration on Glut-4 expression and plasma catecholamines in high sucrose diet rats

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Background and aims: Previous results from our lab have shown that caffeine administration restores insulin sensitivity in rats submitted to a high sucrose (HSu) diet, an effect that was not related to changes in weight gain, visceral fat mass, serum free fatty acids, cortisol nor nitric oxide production. In the present work we have investigated if caffeine reversion of diet-induced insulin resistance was due to an effect on sympathetic nervous system activity, measured as plasma catecholamines, and/or to an altered expression of GLUT-4 in insulin-sensitive tissues.

Materials and methods: Four groups of Wistar rats, aged 9-12 weeks were used. The control group drank regular tap water. The HSu group was administered 35% sucrose in drinking water during 28 days. In each group the animals were randomly divided into two subgroups: treated with 1g/l caffeine during the last 15 days of the experimental protocol and not treated. The insulin tolerance test (ITT) was used to measure insulin sensitivity. Catecholamines were measured in serum by HPLC with electrochemical detection. GLUT-4 expression was determined by Western Blot in skeletal muscle, liver and adipose tissue and normalized to loading protein.

Results: HSu diet induced insulin resistance and hypertension which were prevented by caffeine treatment. HSu diet significantly increased plasma catecholamines by 211.9% from a control value of $50.03 \pm 6.91 \mu$ M ($n = 12$). Chronic caffeine intake did not modify plasma catecholamines in control animals, however when administered together with the HSu diet caffeine prevented the increase in circulating catecholamines suggesting that the metabolic and hemodynamic effects of chronic caffeine intake are mediated by a decrease in sympathetic activation. Moreover, KITT was negatively correlated with plasma catecholamines both in the absence ($r = -0.399$, $p = 0.0484$) and presence ($r = -0.419$, $p = 0.009$) of caffeine treatment. HSu diet decreased

Glut-4 expression in skeletal muscle by 54.9% ($n = 3$). Caffeine administration increases no significantly Glut-4 expression in skeletal muscle by 32.4%, an effect that was insufficient to restore Glut-4 expression to control levels.

Conclusion: The present results suggest that the effects of chronic caffeine administration on insulin resistance and hypertension induced by HSu diet involve a decrease in sympathetic activation and not an alteration of Glut-4 expression in skeletal muscle.

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PS 034 Carbohydrate metabolism

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Pathogenesis of fasting hyperglycaemia in prediabetes

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Background and aims: The exact mechanism of fasting hyperglycemia in individuals with prediabetes remains poorly understood. The present studies were undertaken to determine the cause/s of isolated fasting hyperglycemia in individuals with normal glucose tolerance (IFG/NGT).

Materials and methods: Following OGTT, 14 IFG/NGT and 16 age, sex and BMI matched subjects with NFG/NGT underwent a four hour somatostatin clamp with replacement glucagon and growth hormone. Insulin was infused at 0.35mu/kgTBW/min to maintain plasma glucose in prediabetic range (~6.1mM). Results were compared using averages for last forty minutes of basal and clamp periods using Wilcoxon rank sum tests.

Results: Despite slightly higher fasting insulin (49.0 ± 6.0 vs. 37.0 ± 4.0 pmol/L) and c-peptide (1.1 ± 0.1 vs. 0.77 ± 0.05 nmol/L) concentrations, fasting glucose concentration (6.1 ± 0.1 vs. 5.3 ± 0.1 mM) was significantly higher ($p < 0.001$) in IFG/NGT vs. NFG/NGT. In contrast, clamp glucose (6.3 ± 0.1 vs. 6.0 ± 0.1 mM) and all hormone concentrations were matched between groups ($p = ns$). Fasting endogenous glucose production (EGP) was significantly higher (16.4 ± 0.34 vs. 14.1 ± 0.6 μ mol/kg/min; $p < 0.01$) in IFG/NGT vs. NFG/NGT. On the other hand in the presence of matched glucose and insulin concentrations, EGP (10.5 ± 1.1 vs. 8.5 ± 1.0 μ mol/kg/min) and glucose disappearance (Rd) were no different (29.1 ± 4.6 vs. 34.1 ± 3.4 μ mol/kg/min) in IFG/NGT than NFG/NGT ($p = 0.8$).

Conclusion: In prediabetic individuals with isolated fasting hyperglycemia a) basal EGP is increased but suppresses normally when insulin is increased; b) basal Rd is not decreased and increases normally when insulin is increased; c) the increased EGP in the presence of an abnormal set point for insulin secretion explains the mechanism of increased fasting glucose. This is complementary to our previously published data that concluded that in subjects with isolated IFG, normal EGP and Rd in the presence of insulin concentrations in the postprandial range combined with normal post-prandial insulin secretion results in normal postprandial glucose metabolism.

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Longitudinal multicenter analysis of glucose metabolism development in obese children

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Background and aims: Although there is ample evidence of impaired glucose metabolism in obese children from single cohorts, data are lacking on the progression of glucose metabolism in obese children. We performed a large-scale multicentric longitudinal analysis on glucose metabolism development in obese children.

Materials and methods: Between years 2000 and 2010, 179758 patient visits were documented in the APV registry by 162 central European centers specialized in pediatric obesity. Of the 57988 obese patients, 19.35% (age 12.9 ± 3.0 y, BMI SDS 2.59 ± 0.58 , $n = 10364$) were evaluated by oGTT according to ADA criteria and 1.88% had at least two oGTTs. Patients were stratified for glucose metabolism pathology with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type 2 diabetes (T2D).

Results: A total of 12.6% of the children presented with pathology in glucose metabolism (5.99% IFG, 5.51% IGT, 1.07% T2D). Children with more overt pathology (IGT and T2D) were significantly older, more (visceral) obese, and the percentage of girls was higher. Multivariate analysis identified pubertal group and BMI as significant predictors for impaired glucose metabolism. For the 929 patients with follow-up oGTT (observation interval 1.48 ± 1.32 y), we observed a slight though significant reduction in BMI SDS of 0.094 ± 0.42 . Along with this, mean levels of metabolic parameters improved. While 18.8%

of these patients showed abnormalities in glucose metabolism at baseline, the percentage decreased to 13.7% at follow-up. Of the children with IGT initially, more than 50% converted to normal glucose tolerance. Overall, the oGTT result deteriorated in only 8.5%, while 13.8% improved. The change in BMI SDS predicted improvement in oGTT as well as AUC BG, while sex, age, observation time, age and even BMI SDS at baseline did not have significant impact in multivariate analyses.

Conclusion: We provide evidence for significant improvement of oGTT parameters of obese patients treated in specialized treatment centers, even if reduction in BMI is mild.

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Sources of hepatic glycogen synthesis in healthy subjects following a milk-containing breakfast

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Background and aims: Following a mixed meal, the liver is able to synthesize glycogen from several carbohydrate sources including galactose. A single glass of skimmed milk contains sufficient galactose to contribute significantly to postprandial hepatic glycogen synthesis in healthy subjects. To date, no methods exist to measure this contribution, hence the goal of this study was to further develop the deuterated water (2H₂O) method to measure the contribution of dietary galactose to glycogen synthesis flux in addition to those of the direct and indirect pathways. Glucuronide enrichment from deuterated water (2H₂O) was used to quantify direct and indirect pathway contributions to hepatic glycogen synthesis following a breakfast meal that included 200 ml skimmed milk. Under these conditions, glucuronide position 2 enrichment (G2) was significantly less than that of body water. We hypothesized that incomplete glucose-6-P-fructose-6-P (G6P-F6P) exchange during direct pathway metabolism of glucose and/or inflow of unlabeled UDP-glucose from galactose were responsible. We resolved the exchange and galactose contributions to the reduced G2 enrichment by independently measuring G6P-F6P exchange.

Materials and methods: In Study 1, G6P-F6P exchange in six healthy subjects was quantified by supplementing a milk-containing breakfast meal with 10 grams of [U-2H₇]glucose and quantifying the depletion of position 2 enrichment in urinary menthol glucuronide. In Study 2, another six subjects ingested 2H₂O and Acetaminophen followed by an identical breakfast meal with 10 grams of [1-13C]glucose to resolve direct/indirect pathways and galactose contributions to glycogen synthesis. Glucuronide, glucose and body water 2H/13C-enrichments were determined by 2H- and 13C-NMR.

Results: In Study 1, G6P-F6P exchange approached 100%, therefore the difference between G2 and body water enrichments in Study 2 ($0.20 \pm 0.03\%$ versus $0.27 \pm 0.03\%$, $p < 0.005$) was attributed to galactose glycogenesis. Dietary galactose contributed $19 \pm 3\%$ to hepatic glycogen synthesis. Of the remainder, $58 \pm 5\%$ was derived from the direct pathway and $22 \pm 4\%$ via the indirect pathway.

Conclusion: The contribution of dietary galactose to hepatic glycogen synthesis was resolved from that of direct and indirect pathways using a combination of 2H₂O and [1-13C]glucose tracers. A breakfast meal that included 200 ml of skimmed milk accounted for about one-fifth of postprandial hepatic glycogen synthesis via galactose, a contribution that was comparable to that of the indirect pathway.

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Analysis of glucose enrichment from a double tracer meal tolerance test by LC-MS

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Background and aims: The study of glucose kinetics during meal ingestion provides key insights into insulin-mediated control of postprandial glucose metabolism and can be followed with stable isotope glucose tracers. We

present a simple, rapid and sensitive LC-MS/MS method for direct analysis of glucose tracer enrichments from blood spots. We applied this method to quantify plasma [6,6-2H₂]glucose and [U-13C]glucose enrichments from healthy rats given a meal tolerance test enriched with [6,6-2H₂] and [U-13C] glucose and to evaluate endogenous glucose kinetics.

Methods: Seven 24 hr fasted male Wistar rats were anesthetized and cannulated with an a/v loop at the femoral vein. A primed infusion of [U-13C] glucose was started 60 min before meal delivery and continued throughout the study. A mixed meal containing 5% [6,6-2H₂]glucose was administered intestinally. Blood was periodically sampled from 15 min before to 120 min after the meal by spotting on to filter paper. The dried blood samples were washed with ethanol and passed through a 3K mw cutoff filter and the supernatant evaporated. It was further cleaned by micro solid phase extraction (Stage Tips C18) using acetonitrile and water solvents. Samples were analyzed in triplicate on an Ultimate 3000 LC system coupled to a 4000 QTrap mass spectrometer. Glucose enrichment was quantified using Multiple Reaction Monitoring (MRM). The MRM transitions used were 179/89 for unlabelled glucose, 181/90 and 181/91 for [6,6-2H₂] glucose, 185/92 for [U-13C]glucose and 192/94 for internal standard. Each sample was analyzed using an LC program of 15 min followed by a column cleaning step of 12 min.

Results: The limit of quantification for [U-13C]glucose and [6,6-2H₂]glucose was 0.5 pmol and the method was linear from 0.5-250 pmol/μL glucose concentrations. The mean coefficient of variance was $20.3 \pm 2.3\%$. Figure 1 shows glucose excursions and enrichment profiles before and during the meal tolerance test. Plasma glucose levels doubled within 15 min after the meal, then subsided for the remaining period. From plasma [U-13C]glucose enrichment levels measured before the meal, basal glucose production was estimated to be 70 ± 10 micromol/kg/min. During this period, there was no excess enrichment detected from [6,6-2H₂]glucose. In the initial 15 min after the meal, plasma [6,6-2H₂]glucose rose steeply, reflecting absorption of the meal glucose. At the same time, [U-13C]glucose enrichment declined reflecting dilution by absorbed glucose. The kinetics of meal glucose appearance was superimposable on that of plasma glucose levels indicating that postprandial plasma glucose excursion was tightly coupled to glucose absorption rates.

Conclusions: Plasma glucose enrichments from [U-13C]- and [6,6-2H₂]glucose were quantified by a simple and sensitive LC-MS/MS procedure. This approach was used to characterize systemic and meal tracer appearance profiles in a meal tolerance test.

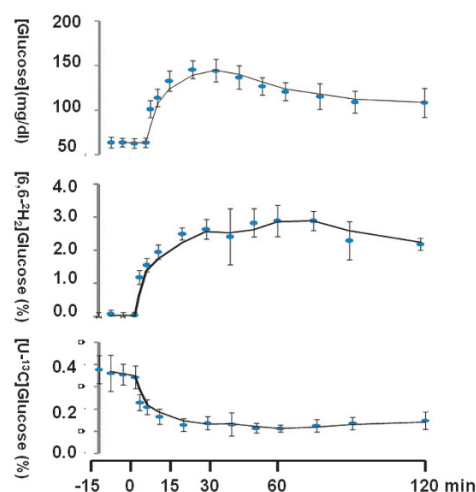


Figure 1: Plasma glucose levels, meal-derived [6,6-2H₂]glucose and infused [U-13C]glucose enrichments 15 min before and 0-120 min after a meal tolerance test.

Clinical Trial Registration Number: PTDC-EEB-BIO-98110-2008

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Triple Tracer (TT) and Double Tracer (DT) techniques are reliable methods to estimate glucose appearance in type 1 diabetes

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Background and aims: Measurement of physiological postprandial glucose fluxes in type 1 diabetes could potentially facilitate improvements in modern insulin therapy regimens. TT technique has been proposed to be the gold standard technique to measure postprandial glucose appearance. We validated TT technique and compared it against DT technique in type 1 diabetes.

Materials and methods: Sixteen young subjects with type 1 diabetes (age 19.5 ± 3.8 yrs, BMI 23.4 ± 1.5 kg/m², HbA1c $8.7 \pm 1.7\%$, diabetes duration 9.0 ± 6.9 yrs, total daily insulin 0.9 ± 0.2 U/kg/day; mean \pm SD) were studied. From 1800 to 0200 next day, intravenous (iv) 20% dextrose enriched with [¹³C]glucose was infused at a variable rate mimicking meal-derived glucose appearance while iv insulin was administered to achieve basal and postprandial insulin concentration. The variable dextrose infusion mimicked a meal-derived glucose appearance of a slowly absorbed meal in the first eight subjects and a fast absorbed meal in the second eight subjects. From 1530 to 0200, primed iv [6,6-²H]₂glucose was infused in a manner that mimicked the expected endogenous glucose production. From 1800 to 0200, iv [¹³C; 1,2,3,4,5,6,7-²H]₇glucose was infused in a manner that mimicked the expected glucose appearance from a standard meal. The iv dextrose infusion was reconstructed using TT and DT techniques utilizing a modified stochastic Mari model. Plasma glucose was measured every 10–15 min. Glucose enrichment was measured by gas chromatography – mass spectrometry every 10–30 min.

Results: Figure shows actual and reconstructed dextrose infusion rates. The difference between individual actual and individual reconstructed dextrose infusion rates as assessed by the root mean square error (RMSE) was identical for the two methods (6.6 ± 1.9 vs. 7.98 ± 3.5 μ mol/kg/min; TT vs. DT; $P = \text{NS}$, paired t-test). RMSE did not differ when dextrose infusion mimicked a fast or a slow meal (6.96 ± 1.5 vs. 6.24 ± 2.0 μ mol/kg/min for TT and 6.81 ± 2.2 vs. 9.15 ± 4.3 μ mol/kg/min for DT; fast vs. slow; $P = \text{NS}$, unpaired t-test). RMSE associated with mean dextrose infusion was 2.5 and 3.3 μ mol/kg/min for TT and DT, respectively. Overall, $100 \pm 9\%$ and $92 \pm 12\%$ ($P = 0.02$) of the dextrose infusion was recovered. There was no difference in dextrose recovery when fast meal or slow meal mimicking dextrose infusion was used.

Conclusion: TT and DT techniques combined with advanced computational methods can measure reliably postprandial glucose appearance in type 1 diabetes. TT tends to outperform slightly DT but the latter benefits from reduced experimental and analytical complexity.

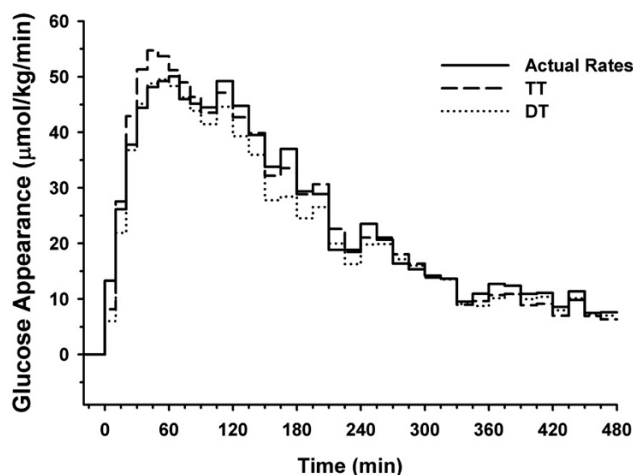


Figure. Actual infusion rate of dextrose and reconstructed infusion rate using triple tracer (TT) and double tracer (DT) techniques (N=16, mean is shown).

Supported by: JDRF, NIHR and Diabetes UK

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Estimating gluconeogenesis from position 3 enrichment of plasma glucose by deuterated water: avoidance of transaldolase exchange effects

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Background and aims: Deuterated water 2H₂O is widely used to measure the gluconeogenic (GNG) contribution to endogenous glucose production (EGP) in humans by analysis of plasma glucose enrichment in position 5 (H5). We recently showed that transaldolase exchange (TA) accounts for ~35% of H5 resulting in GNG overestimation. TA does not influence the enrichment of hydrogen 3 (H3) from 2H₂O. H3 originates from triose phosphate isomerase (TPI) exchange and assuming that a) TPI exchange is complete and b), 2H incorporation by this mechanism is not subject to isotopic discrimination, then H3 reflects GNG flux and is insensitive to TA. We hypothesized that H3 provides equivalent estimates of GNG contributions to those derived from H5 following correction for TA. If so, then GNG fluxes can be directly derived by analysis of H3 from 2H₂O without the need for TA correction with additional tracers.

Materials and methods: 2H₂O, [3-¹³C]glucose and [1-¹³C]acetate were administered to 16 subjects with impaired fasting glucose/impaired glucose tolerance (IFG/IGT) and 14 age and BMI matched normal fasting glucose/normal glucose tolerance (NFG/NGT) subjects. Glucose enrichment from both tracers was measured by 2H and 13C NMR following an overnight fast and during a 0.35 μ U/kgFFM/min insulin infusion. Somatostatin, glucagon and growth hormone were infused during the clamp to ensure comparable and equal portal concentrations in both groups. The fraction of hepatic G6P that underwent TA was estimated from the distribution of 13C-enrichment in positions 3 and 4 of glucose. H5 was then corrected for the fraction derived by TA and GNG flux was estimated from this adjusted value (TA-adjusted). These estimates were then compared to GNG fluxes derived from H3 measured directly from the 2H NMR spectrum (Figure 1).

Results: Under basal conditions, fasting EGP was 14.1 ± 0.6 μ mol/kg/min. The GNG contribution (TA-corrected) was 5.0 ± 0.3 μ mol/kg/min while that estimated directly from H3 was 4.3 ± 0.3 μ mol/kg/min ($p = 0.30$) for the NFG/NGT group. During the clamp, EGP was 8.5 ± 1.0 μ mol/kg/min. GNG (TA-corrected) was 2.3 ± 0.3 μ mol/kg/min versus 2.4 ± 0.3 μ mol/kg/min derived by H3 analysis ($p = 0.93$). For the IFG/IGT group, basal EGP was significantly higher than control values (16.7 ± 0.3 μ mol/kg/min, $p < 0.001$). The GNG contribution (TA-corrected) was 5.7 ± 0.3 μ mol/kg/min versus 4.6 ± 0.3 μ mol/kg/min estimated directly from H3 ($p = 0.06$). During clamp EGP was 13.0 ± 1.1 μ mol/kg/min, significantly higher compared to controls ($p < 0.005$). The TA-corrected GNG contribution was 4.1 ± 0.4 μ mol/kg/min while H3 analysis yielded a value of 3.6 ± 0.4 μ mol/kg/min, ($p = 0.47$).

Conclusion: Glucose H3 can be directly measured by 2H NMR analysis of the monoacetone glucose derivative following 2H₂O ingestion precluding the requirement for additional tracer such as [1-¹³C]acetate to correct for transaldolase exchange.

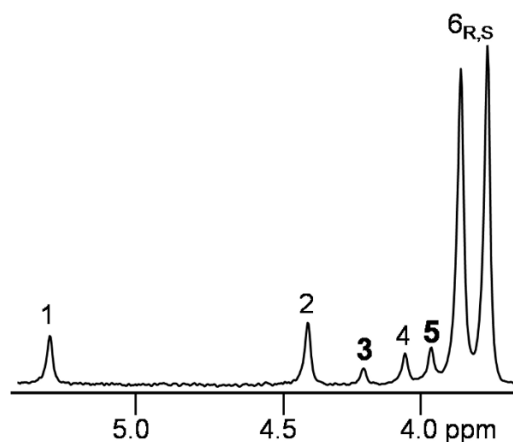


Figure 1: ²H NMR spectrum of the monoacetone derivative of plasma glucose. The numbers above each signal represent their position in the glucose molecule and positions 3 and 5 are shown in bold.

Clinical Trial Registration Number: NIDDK29953

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Bed rest as a model for immobility induced insulin resistanceM. Heer^{1,2}, S. Wnendt³, P. Frings-Meuthen²;¹Nutritional Sciences, Profil Institute for Metabolic Research, Neuss, Germany, ²Space Physiology, German Aerospace Center (DLR), Cologne, ³MLM Medical Labs Moenchengladbach GmbH, Germany.

Background and aims: Physical inactivity decreases insulin sensitivity. In general, however, glucose and insulin levels show large variability. We therefore kept nutrient intake constant on a day-by-day level and used bed rest as a model for immobility. With this set up of highly standardized study conditions we wanted to demonstrate that 7 test subjects are sufficient to achieve significant differences in plasma glucose and insulin concentrations and hypothesized that 4 days of light physical workload will reverse the immobility induced impaired glucose tolerance.

Materials and methods: Seven healthy male volunteers (age: 27.6 ± 3.3 years (mean \pm SD); body mass: 78.6 ± 6.4 kg; height: 1.81 ± 0.04 m; BMI: 24.1 ± 1.9 kg/m²) participated in a randomized crossover study of 21 days head-down tilt bed rest. The volunteers stayed twice for the entire period of 28 days (7 days of adaptation, 21 days bed rest and 7 days recovery) in a metabolic ward. Nutrient intake was individually tailored and kept constant on a day-by-day basis. An oral glucose tolerance test was applied four days before, on day 21 of bed rest and 5 and 14 days after bed rest.

Results: After 21 days of bed rest, the test subjects had developed impaired glucose tolerance (plasma glucose, before: basal: 91 ± 4 mg/dL, after 120 min: 96 ± 12 mg/dL; day 21 of bed rest: basal: 92 ± 6 mg/dL, after 120 min: 145 ± 21 mg/dL $p < 0.001$). In addition, circulating insulin concentrations were increased compared to before bed rest (before: basal: 6.6 ± 1.7 mU/L, after 120 min: 22.7 ± 11.4 mU/L, day 21 of bed rest: basal: 6.7 ± 1.7 mU/L, after 120 min: 52.9 ± 28.1 mU/L; $p < 0.001$). Area under the curve (AUC) determinations for glucose were significantly increased on day 21 of bed rest ($p = 0.002$) and were back to their pre-bed rest level only after 14 days of recovery. While insulin resistance analyzed by HOMA IR index did not change, insulin sensitivity estimated by ISI_{composite} index was reduced during bed rest.

Conclusion: We conclude from these results that it takes up to 14 days of light physical workload to reverse impaired glucose tolerance in healthy young subjects induced by bed rest.

Supported by: ESA Human Life Sciences

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Biphasic relationship between glucose tolerance and gastric emptying in healthy subjectsC.S. Marathe^{1,2}, M. Horowitz^{1,2}, C.K. Rayner^{1,2}, K. Lange^{1,2}, K.L. Jones^{1,2};¹University of Adelaide, Discipline of Medicine, Royal Adelaide Hospital,²Centre for Clinical Research Excellence in Nutritional Physiology, Interventions and Outcomes, University of Adelaide, Australia.

Background and aims: The rate of gastric emptying (GE) is recognized as a major determinant of the magnitude of the initial rise (e.g. at 30min) in blood glucose following oral glucose (75g), or carbohydrate-containing meals in both health and type 2 diabetes i.e. when GE is faster, the increase in glucose is greater. In health, GE of glucose is highly reproducible and tightly regulated at a rate of 1–4 kcal/min and it has, accordingly, been suggested that relatively more rapid GE may predispose an individual to the development of type 2 diabetes. There is, however, little information about the impact of GE on blood glucose 120min after a 75g oral glucose load, which is used diagnostically. Moreover, recent studies employing direct infusion of glucose into the duodenum indicate that the relationship between the glycaemic response and GE is non-linear. We investigated the impact of GE on the magnitude of the glycaemic excursion and blood glucose 120min after a 75g oral glucose load in health.

Materials and methods: 52 healthy subjects aged 18–68 years, who each drank 350ml water containing 75g glucose and 20MBq 99mTc-sulphur colloid while sitting in front of a gamma camera, were studied. GE data were acquired for at least 120min and the 50% GE time (t50) calculated. Blood glucose was measured immediately before ($t = -2$) and at 15, 30, 60 and 120min following consumption of the glucose drink. Relationships between blood glucose and GE were evaluated using linear and non-linear models. Data are shown as mean \pm SEM.

Results: In all subjects GE approximated an overall linear pattern. The rate at which glucose emptied from the stomach (based on t50) was 1.52 ± 0.07 kcal/min. The blood glucose at 30min ($r = -0.33$, $P < 0.02$; Figure A),

and the change in blood glucose from baseline at 30min ($r = -0.28$, $P < 0.05$), were inversely related to the t50. In contrast, the blood glucose at 120min ($r = 0.40$, $P < 0.005$; Figure B) was related directly to the t50. Analysis of the relationship between the change in blood glucose at 30min with t50 demonstrated that a non-linear relationship, such as an exponential association ($r = 0.29$, $P < 0.05$) or S curve ($r = 0.30$, $P < 0.02$), were as compatible with the data as a linear relationship - both non-linear models showed an initial increase in glycaemia that slowed at higher rates of GE.

Conclusion: In healthy subjects, the relationship between glycaemia and GE of a 75g glucose load is 'biphasic', so that the initial rise is greater, and the blood glucose at 120min less, when GE is relatively more rapid.

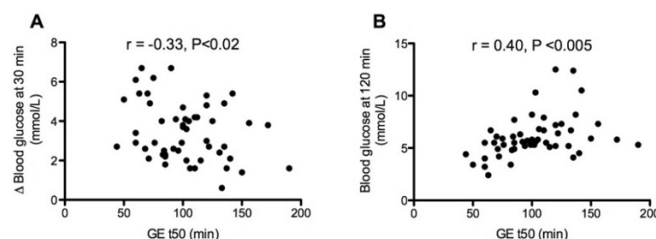


Figure: Inverse relationship between the magnitude of the rise in blood glucose from baseline at 30min and the 50% emptying time (t50) (A) and direct relationship between the blood glucose concentration at 120min and t50 (B), following a 75g oral glucose load in healthy subjects ($n = 52$).

Supported by: National Health and Medical Research Council, Australia

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Simple and convenient alternatives to the mixed meal tolerance test

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Background: Mixed meal tolerance test (MMTT) stimulated serum C-peptide (SCP) is a gold standard measure of endogenous insulin secretion capacity but practical difficulties limit its clinical use. Urinary C-peptide creatinine ratio (UCPCR) allows assessment of C-peptide on a spot urine sample and is stable for 3 days in a standard mid stream urine container.

Aims: 1. Can UCPCR replace serum C-peptide in the MMTT. 2. Can home post meal UCPCR replace serum C-peptide in the MMTT

Materials and methods: We performed standardised MMTTs in a mixed group of 103 individuals with insulin treated diabetes (59 Type 1). We measured fasting and 90 minute post MMTT SCP and fasting and 2 hour post MMTT UCPCR. In addition all patients were asked to provide a urine sample 2 hours following their largest meal at home.

Results: MMTT UCPCR was highly correlated with Serum C-Peptide ($r = 0.92$, $p < 0.001$) and remained an excellent test for severe insulin deficiency (MMTT SCP < 0.2 nmol/L): using an optimal cut off of 0.47 nmol/mmol, giving 100% sensitivity and 95% specificity. Home UCPCR was also highly correlated with the gold standard serum C-Peptide ($r = 0.84$, $p < 0.001$) an optimal cut off 0.12 nmol/mmol with 92% sensitivity and 98% specificity.

Conclusion: UCPCR after a home meal is a reliable measure of endogenous insulin secretion which will allow practical assessment of beta cell function in primary care and the outpatient setting.

PS 035 Metabolic effects of bariatric surgery

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Comparison of the effectiveness of bariatric surgery in type 2 diabetes according to the surgical procedure: retrospective analysis in 74 diabetic operated patients

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Background and aims: French recommendations allow bariatric surgery as a treatment for morbid obesity (BMI > 40 kg/m²) or obesity (BMI > 35 kg/m²) associated with comorbidities including diabetes. Overall, the different published studies have reported a remission of diabetes in approximately 80% of patients after bariatric surgery. However, results vary as a function of the surgical procedure

Materials and methods: Among 970 patients who underwent bariatric surgery at our University Hospital since 2001, 74 patients were identified as type 2 diabetes (ICD Coding). Laparoscopic adjustable gastric banding (AG), intervention type Mason (MA), gastric bypass (BP) or sleeve-gastrectomy (SG) were performed respectively in 25%, 17%, 28% and 30% of this diabetic population. Clinical follow-up (weight, blood pressure, sleep apnea) and biological (blood glucose, HbA1c, lipid profile) was performed for 3±0.3 years after surgery. The results obtained in diabetic patients were compared to those of a population of 74 non diabetic obese matched for age, sex, BMI and surgical procedure.

Results: Weight loss was significantly lower in diabetic than in non-diabetic patients, except after SG which trend to induce a larger weight loss in diabetic group. SG appeared more effective than AG on HbA1c one year after operatively (-1.6% vs. -1.05%), similar results were obtained after BP (HbA1c -2.06%). The resolution rate of diabetes at 1 year was significantly ($p < 0.01$) higher after SG than AG: 62.5% vs 20%, but not significantly different between SG and BP (62.5% vs. 52.1%, $p = 0.5$). The evolution of diabetes evaluated using an index integrating the changes in HbA1c and in the modalities of the treatment after surgery showed that SG is followed by a statistically significant ($p < 0.002$) better improvement than AG. In terms of LDL-cholesterol reduction BP = SG > MA > AG ($p < 0.03$). For the evolution of the other cardiovascular risk factors, there is no significant difference according to surgical procedures.

Conclusion: These results suggest that SG may be the preferred surgical procedure for the management of type 2 diabetes obese patients.

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Gastric bypass induces a more pronounced improvement in postprandial metabolism and the indices of cardiac function than sleeve gastrectomy

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Background and aims: Bariatric surgery is the most effective long-term treatment for morbid obesity. Roux-en-Y Gastric Bypass (GB) and Sleeve Gastrectomy (SG) are two of the most commonly applied bariatric modalities. The aim of the present study was to compare the effect of these procedures on postprandial glycemia and triglyceridemia as well as on cardiac function, since relevant data are scarce.

Materials and methods: Twenty two patients who underwent either GB (n=9) or SG (n=13), matched for age and BMI, were examined before, three and six months after surgery. A test meal was consumed, consisting of 200 ml ice cream (450 kcal). Blood samples were collected before meal initiation and every 30 minutes until 180 minutes postprandially. Differences in glucose and triglycerides were examined at different postprandial time points, as well as in terms of area under the curve divided by time (AUC) using the trapezoid rule. Epicardial fat thickness, aortic distensibility, left ventricular Tei index and left atrium diameter were assessed by cardiac ultrasonography before and six months after surgery in a subset of these patients (GB: 8, SG: 10).

Results: Both groups experienced significant ($p < 0.001$) and similar weight loss (BMI reduction at three months GB: -19.4% vs SG: -18.0%, $p = 0.57$, at six months GB: -26.9% vs SG: -26.1%, $p = 0.71$). Three months after surgery blood glucose levels were significantly lower in GB patients at 120 minutes postprandially compared to SG patients (GB: 92.2 ± 8.8 vs SG: 101.2 ± 9.7 mg/dl, $p = 0.04$). Triglyceride levels were also significantly lower in the GB group at 60, 90, 150 and 180 minutes postprandially ($p = 0.03, 0.01, 0.03$ and 0.01 respectively). The same was true for the triglyceride AUC (GB: 109.0 ± 26.6 vs SG: 144.4 ± 38.6 mg/dl, $p = 0.03$). Six months after surgery blood glucose levels were significantly lower in the GB group at 60, 90 and 120 minutes postprandially compared to those of the SG group ($p = 0.04, 0.02$ and 0.04 respectively), as well as in terms of AUC (GB: 86.6 ± 11.5 vs 99.3 ± 11.5 mg/dl, $p = 0.04$). Triglyceride levels were also significantly lower in the GB group both at all separate time points ($p < 0.01$), and when expressed as AUC (GB: 78.7 ± 19.2 vs SG: 123.8 ± 27.8 mg/dl, $p = 0.003$). Epicardial fat and Tei index were comparable in both groups preoperatively (ep. fat GB: 1.62 ± 0.09 vs SG: 1.61 ± 0.13 mm, $p = 0.8$, Tei GB: 0.45 ± 0.01 vs SG 0.46 ± 0.02, $p = 0.4$), and decreased to a greater extent in the GB group 6 months postoperatively (ep. fat GB: 1.38 ± 0.08 vs SG: 1.50 ± 0.07 mm, $p = 0.005$ and Tei GB: 0.41 ± 0.01 vs 0.43 ± 0.01, $p = 0.04$). No pre- or postoperative differences were found in aortic distensibility and left atrium diameter.

Conclusion: Gastric bypass resulted in better postprandial glucose and triglyceride responses 3 and 6 months after surgery, compared to sleeve gastrectomy, as well as in greater improvement in some of the ultrasonographic indices of cardiac function. This different cardiometabolic profile implies a role for the differences in the hormonal milieu produced by these procedures.

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Effect of bariatric surgery induced weight loss on hepatic glucose uptake

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Background and aims: Weight loss improves whole body insulin sensitivity, but its mechanisms are not well understood. The aim of this study was to investigate the effect of massive weight loss on hepatic insulin sensitivity.

Materials and methods: A total of 23 morbidly obese, eight non-diabetic and 15 diabetic or pre-diabetic patients, were studied before and six months after a bariatric surgery (either sleeve gastrectomy (n=10) or gastric bypass (n=13) surgery). Ten healthy controls (BMI 23.7 ± 1.8 kg/m²) were included. Hepatic glucose uptake (GU) was measured using positron emission tomography and ¹⁸F-fluorodeoxyglucose during euglycemic hyperinsulinemic clamp. Liver volume and fat content were measured using magnetic resonance imaging and spectroscopy.

Results: BMI decreased postoperatively by 25% (from 43 ± 4 to 32 ± 3 kg/m²) in diabetic and by 20% (from 44 ± 4 to 35 ± 5 kg/m²) in non-diabetic patients, both $p < 0.001$ vs. baseline. Abdominal visceral fat mass was reduced by 1.8 ± 1.1 kg ($p < 0.001$) and 1.2 ± 0.5 kg ($p = 0.001$), respectively. Whole-body insulin mediated glucose uptake (M value) was significantly impaired both in diabetic (11.8 ± 5.2 μmol/min/kg) and in non-diabetic patients (14.0 ± 7.0 μmol/min/kg) compared to controls (40.3 ± 9.5 μmol/min/kg), both $p < 0.001$, and increased by 119 % ($p < 0.001$) in diabetic and by 81 % in non-diabetic patients ($p = 0.001$) after surgery. OGTT was normalized in 10 out of 13 patients after follow-up. Diabetic subjects had lowest insulin stimulated hepatic GU at baseline, which increased significantly postoperatively (from 1.6 ± 0.5 to 2.1 ± 0.9 μmol/100ml/min, $p = 0.016$). No significant change was observed in hepatic GU in non-diabetic subjects. Also no group differences were observed in hepatic GU postoperatively. Postoperative hepatic GU results were similar between the two surgically treated groups (sleeve gastrectomy vs. gastric bypass). Liver volume decreased by 29 % in diabetic (from 1900 ± 334 to 1333 ± 266 ml $p < 0.001$) and by 15 % in non-diabetic patients (from 1747 ± 363 to 1452 ± 227 ml $p = 0.044$) but remained still higher than in healthy controls (1115 ± 99 ml) in both groups (diabetic vs. control group: $p = 0.023$ and non-diabetic vs. control group: $p < 0.001$). Liver fat content was reduced postoperatively in diabetic patients (from 12.0 ± 7.7 to 2.5 ± 1.4 % $p = 0.002$) but only a tendency was observed in non-diabetic subjects (from 8.5 ± 8.6 to 2.8 ± 2.8 % $p = 0.06$). No group differences were found in liver fat content postoperatively (1.9 ± 1.2 %, control subjects). Hepatic GU correlated positively with the M value through the study and (preoperatively) inversely

with WHR, liver volume, visceral fat and TNF- α . After bariatric surgery, a negative correlation was found between hepatic GU and WHR, liver fat and visceral fat, and a positive correlation between hepatic GU and ghrelin and adiponectin.

Conclusion: In conclusion, bariatric surgery normalized the excess in liver fat content and the defect in insulin-mediated hepatic GU in morbidly obese patients with diabetes and pre-diabetes. No difference was observed between sleeve gastrectomy and gastric bypass surgery.

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Early aggressive weight loss efforts using adjustable gastric banding leads to “remission” or improvement of type 2 diabetes mellitus

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Background and aims: Bariatric surgery (malabsorptive or restrictive techniques) has been established as an effective treatment to reduce weight in severely obese patients' refractory to behavioral and medical therapies. This study reports the 1 year “remission” and/or improvement of type 2 diabetes mellitus (T2D) after laparoscopic placement of the adjustable gastric band (AGB) as documented by T2D medication reduction/discontinuation, and the accompanying change in BMI as well as improvements in other co-morbidities of obesity and quality of life.

Materials and methods: The APEX study is an ongoing 5-year, prospective, multi-center, open-label, observational study to assess weight reduction, co-morbidities and quality of life after implantation of the LAP-BAND AP[®] gastric band, a restrictive weight loss technique. This is an interim analysis of subjects who reported daily medical therapy for T2D before AGB and who have completed the 1 year post-operative scheduled visit. “Remission” of diabetes was defined as elimination of hypoglycemic medication, and “improvement” as reduction in hypoglycemic medication.

Results: At baseline, 94 out of 436 subjects (22%) reported T2D requiring daily medical therapy; data from 64 subjects contained sufficient information to assess outcome at 48 weeks. Overall, 86% had “remission” and/or improvement in T2D, with remission more likely to occur in patients treated earlier after the diagnosis of T2D (as shown in table below).

		Remission	Improvement	Stable	Worse
% (n)		34 (22)	52 (33)	13 (8)	2 (1)
Mean	Duration T2D (mo)	63	76	90	1
	Baseline BMI	46	44	52	47
	Δ BMI	-8.9	-7.6	-8.1	-2.9
	Δ Wt (kg)	-25	-22	-24	-8
	% Wt Δ	-19	-21	-15	-6

Baseline BMI, reductions in BMI and % change in weight were not statistically different among the groups, although numbers were small. As in patients with T2D, resolution or improvement also occurred in other pre-existing co-morbidities measured: hypertension (78%), hyperlipidemia (57%), depression (71%), obstructive sleep apnea (69%) and GERD (93%). Quality of Life as measured by the Obesity and Weight Loss Quality of Life instrument also improved.

Conclusion: These data suggest that a minimally-invasive restrictive gastric banding procedure in obese patients with T2D results in clinically meaningful weight loss, as well as a reduction in T2D medication requirements, likely without the risk of nutrient deficiencies often seen with malabsorptive surgery. An earlier, aggressive weight loss intervention appears more apt to facilitate remission of disease, probably due to improvements in insulin sensitivity and beta cell health. Larger and longer-term studies are required to determine the duration of remission/improvement and the effects on beta cell function.

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Effects of gastric bypass surgery on glucose metabolism 5 days, 3 months and 1 year after surgery in subjects with type 2 diabetes and normal glucose tolerance

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Background and aims: Roux-en-Y gastric bypass (RYGB) has profound effects on glucose metabolism leading to a resolution of type 2 diabetes (T2D) early after surgery that persists for years; however, the mechanisms for this improvement remain uncertain.

Materials and methods: 13 obese T2D (BMI: 43.1 \pm 1.4 kg/m²) and 12 matched normal glucose tolerant (NGT) subjects were examined during a liquid meal 3 days before, 5 days, 3 months and 1 year after RYGB. Three fasting blood samples were drawn before the meal (Fresubin Energy, 1260 kJ, 200 mL, protein E% 15, carbohydrate E% 50, fat E% 35), followed by repeated blood sampling for 4 hrs for measurement of plasma glucose, insulin and C-peptide. Insulinogenic index was calculated as Δ C-peptide/ \rightarrow peak glucose-fasting glucose / Δ glucosepeak-fasting. To relate beta-cell function to the ambient insulin resistance, disposition index was calculated as the insulinogenic index multiplied by 1/HOMA-IR.

Results: A significant decrease in fasting glucose (T2D: pre: 8.8 \pm 0.6, post: 7.0 \pm 0.3 p<.01, 3 mo: 6.8 \pm 0.5 p<.01, 1y: 6.8 \pm 0.75 mmol/l p<.05; NGT: 5.5 \pm 0.2, 5.0 \pm 0.2 p<.01, 4.9 \pm 0.1 p<.01, 4.9 \pm 0.12 mmol/l) as well as fasting insulin levels (T2D: 125 \pm 21, 73 \pm 9 p<.01, 58 \pm 10 p<.01, 48 \pm 10 pmol/l p<.01; NGT: 82 \pm 8.2, 49 \pm 4 p<.01, 43 \pm 4 p<.01, 34 \pm 4 pmol/l p<.05) was observed after RYGB. HOMA-IR was halved in both groups after 5 days, followed by further non-significant falls by 3 mo and 1y (T2D: 6.6 \pm 1.0, 3.2 \pm 0.4 p<.01, 2.1 \pm 0.4 2.1 \pm 0.4 p<.01; NGT: 2.9 \pm 0.3, 1.6 \pm 0.2 p<.01, 1.2 \pm 0.2, 1.1 \pm 0.15). Following meal ingestion, 120 min post prandial glucose levels decreased in T2D subjects (11.4 \pm 0.8, 8.2 \pm 0.7 p<.01, 6.9 \pm 0.6 p<.01, 7.1 \pm 0.8 p<.01 mM), whereas levels were unchanged in NGT subjects immediately after RYGB, but decreased by 3 mo and 1 y (5.6 \pm 0.3, 5.2 \pm 0.2, 4.2 \pm 0.3 p<.01, 4.3 \pm 0.1 mM p<.01). Incremental AUC C-peptide was increased directly after surgery in the T2D group (188 \pm 21, 254 \pm 28 p<.05, 228 \pm 25, 171 \pm 24 nM min), whereas levels in the NGT group were significantly increased 5 d and 3 mo after, but had returned to pre surgical levels by 1y (168 \pm 17, 262 \pm 28 p<.01, 329 \pm 41 nM min p<.01, 176 \pm 19 nM min). IGI increased after RYGB in T2D, but did not change in NGT, whereas disposition index increased in both groups 5 d and 3 mo after surgery. After 1 y, disposition index continued to increase in the T2D group, but returned towards normal in the NGT subjects (T2D: 52 \pm 11, 119 \pm 12 p<.01, 164 \pm 30 p<.01, 220 \pm 72 p<.05; NGT: 415 \pm 68, 686 \pm 109 p<.05, 848 \pm 107 p<.01, 719 \pm 158).

Conclusion: Resolution of T2D after RYGB is associated with increased insulin sensitivity and beta cell function. Effects are seen as early as 5 days after surgery and persist for at least 1 year. Beta cell function of NGT subjects increase dramatically within the first months after RYGB, but after 1y, beta-cell function has adapted to the ambient insulin sensitivity.

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The effect of metabolic surgery on beta cell function, hepatic insulin extraction, insulin sensitivity and post-hepatic insulin appearance in patients with morbid obesity

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Background and aims: The hormonal and metabolic changes following weight loss by metabolic surgery are of great interest, since it is still unclear why a resolution of type 2 diabetes is seen in the majority of patients. In addition, to changes of gastrointestinal hormones, amelioration of insulin action and beta cell function could play an important role. Since detailed integrated information is still lacking, we have evaluated beta cell function, hepatic insulin extraction, insulin sensitivity and post-hepatic insulin appearance in patients with morbid obesity (MO) before and after metabolic surgery (MS).

Materials and methods: We studied 67 patients with MO (BMI 45.1 ± 0.8 kg/m²; 40.3 ± 1.2 years) at baseline and at 2 years after MS (mean weight loss 40 kg). An oral glucose tolerance test (OGTT; 75 g glucose) was performed before and after MS, as well as in 30 nondiabetic control (CO) subjects (mean BMI 28.4 ± 1.4 kg/m²) matching for age and sex. In the fasting conditions, glucose (Gb; mg/dl), insulin (Ib; μ U/ml), C-peptide (CPb; pmol/ml) yielded beta cell function ($FBC = CPb/Gb$) and insulin sensitivity (QUICKI). From OGTT (post-prandial condition), we calculated the areas under glucose (AUCg; mol/L 2h) and insulin (AUCi; nmol/L 2h) concentration which yielded beta cell (pre-hepatic) function (IGI; pmol/mmol) and hepatic insulin extraction (HE,%). Modeling analysis provided systemic insulin sensitivity (OGIS, ml/min/m²) and the adaptation index (AI) that is the ability of the beta cell to compensate for increasing insulin resistance (also called: beta cell sensitivity to changes in insulin sensitivity).

Results: All data evaluated in the MO patients before and after MS at both fasting and dynamic conditions are summarized in the TABLE. Insulin action markedly ameliorated both at fasting and in dynamic conditions. This is demonstrated also by the marked improvement of the adaptation index, showing that the beta cell responds better to the changes in insulin resistance. Changes in peripheral insulin are due to pancreatic function (secretion) and not to changes in clearance, because hepatic extraction does not change significantly.

Conclusion: Our results show that surgery entails lower glucose levels, which cause a reduction of released insulin, despite a clear increase of the beta cell function. This implies that the pancreas is able to better respond to the glycemic stimulus, as observed when the beta cell is less stressed (less glucotoxicity).

Table 1. Pre- and post-surgery values of fasting and dynamic conditions

parameter	Pre-surgery	Post-surgery	p-value
Gb	97 ± 3	80 ± 2	<0.000001
Ib	26 ± 2	10 ± 1	<0.000001
CPb	1.2 ± 0.1	2.0 ± 0.5	0.011
FBC	0.23 ± 0.02	0.47 ± 0.13	0.0008
QUICKI	0.359 ± 0.004	0.441 ± 0.010	<0.000001
AUCg	0.86 ± 0.02	0.73 ± 0.02	<0.000001
AUCi	82 ± 4	50 ± 6	0.00017
IGI	5.4 ± 0.4	9.1 ± 1.3	0.0018
OGIS	318 ± 8	440 ± 15	<0.000001
HE	50 ± 5	58 ± 10	0.44
AI	1.70 ± 0.14	4.09 ± 0.71	0.00007

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Mechanisms of improvement of glucose in type 2 diabetes after bariatric surgery: gastric bypass vs sleeve gastrectomy

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Background and aims: In morbidly obese patients with T2DM, Roux-en-Y-gastric-bypass surgery (RYGB) restores euglycemia early after surgery, but effectiveness of sleeve gastrectomy (SLV) in improving T2DM are scarce. To investigate extent and mechanisms of recovery of β -cell function and insulin-sensitivity in severely obese T2DM patients undergoing RYGB or SLV.

Materials and methods: 28 obese T2DM subjects (19 RYGB and 9 SLV) were studied before and 15-d after surgery by comparing the response to a Mixed-Meal-Test (MMT) preceded by a week of low-calorie intake. Insulin-sensitivity was assessed by OGIS-index and β -cell function by modeling analysis of the C-peptide response to MMT. Plasma ghrelin concentrations were assessed during MMT.

Results: 15-d post-surgery, BMI had decreased to the same extent in RYGB and SLV (43.5 ± 6.1 vs 40.3 ± 5.6 ; 47.6 ± 5.2 vs 45.5 ± 7.5 kg.m⁻² respectively, $p < 0.0001$ vs baseline). Mean glucose improved in RYGB and SLV (8.2 ± 1.9 vs 7.0 ± 1.8 mmol/l, 8.8 ± 2.1 vs 6.9 ± 1.5 respectively, $p = 0.0002$ vs baseline) and mean insulin decreased (177 ± 64 vs 141 ± 76 pmol/l, 247 ± 130 vs 184 ± 79 , respectively, $p = 0.005$ vs baseline). β -cell glucose sensitivity improved in RYGB and SLV (28.8 ± 22.9 vs 47.9 ± 35.5 pmol.min⁻¹.m⁻².mM⁻¹, 33.0 ± 30.9 vs 48.8 ± 39.5 , respectively, $p = 0.02$ vs baseline). At baseline, insulin-sensitivity

was similar in RYGB and SLV (308 ± 53 vs 289 ± 39 ml.min⁻¹.m⁻²); following surgery, insulin-sensitivity improved (359 ± 53 and 354 ± 68 ml.min⁻¹.m⁻², respectively, $p = 0.0003$ vs baseline). Mean ghrelin decreased in RYGB and SLV (7643 ± 7871 vs 3630 ± 2848 , 4845 ± 5712 vs 1687 ± 782 pg/ml, $p = 0.02$). Plasma ghrelin changes were inversely correlated with β -cell glucose sensitivity variations ($r^2 = 0.096$, $p < 0.03$). After surgery, plasma PYY increased in GBP only (18.98 ± 8.18 vs 24.32 ± 8.57 , 14.42 ± 2.14 vs 14.59 ± 3.81 ng/ml, $p = 0.004$ in GBP and sLV respectively).

Conclusions: 15-d after surgery and under constant calorie intake, glucose tolerance is improved to a similar extent with RYGB and SLV, as a result of similar improvements in β -cell function and insulin-sensitivity. Furthermore, plasma ghrelin concentrations were reduced after both surgery and, correlated with β -cell glucose sensitivity.

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PTH and vitamin D in morbid obese subjects: the effects of type 2 diabetes and roux en-y gastric bypass (RYGB)

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Background and aims: Several lines of evidence indicate that vitamin D (25OH-D) deficit and hyperparathyroidism (hyperPTH) occur after bariatric surgery; some studies have reported that in morbid obesity vitamin D deficit may precede surgery. Furthermore, low 25OH-D and high PTH concentrations have been suggested to be independent risk factors for type 2 diabetes (T2DM). However, data regarding the effect of RYGB in morbidly obese diabetic subjects are scarce. Aim of this study was to investigate the relationship between 25OH-D, PTH concentrations and T2DM in severely obese patients before and after RYGB.

Materials and methods: 130 morbidly obese subjects wait-listed for RYGB (44 non-diabetic (NGT), 28 with impaired glucose tolerance (IGT) and 58 with T2DM) were studied before surgery. Among them, 50 subjects (24 NGT and 26 T2DM) were re-studied 3 months after RYGB. Serum PTH and 25OH-D were measured, insulin sensitivity was assessed by the OGTT-derived OGIS method.

Results: At baseline, BMI was similar in NGT, IGT and T2DM (45.5 ± 7.6 vs 47.4 ± 6.5 vs 48.82 ± 7.8 kg/m², mean \pm SD). In T2DM compared to NGT, PTH concentrations were significantly higher (79.5 ± 36.8 vs 70.7 ± 26.9 vs 66.8 ± 29.7 pg/ml in T2DM, IGT, NGT, respectively, mean \pm SD, $p = 0.05$), whereas 25OH-D levels were significantly lower (11.9 ± 7.3 vs 14.7 ± 12.9 vs 19.0 ± 12.7 ng/ml in T2DM, IGT, NGT, mean \pm SD, $p < 0.03$). Basal PTH was directly related with HbA1c ($p = 0.002$), but not with BMI; 25OH-D did not correlate with BMI or glycaemic control. Following surgery, BMI decreased similarly in both NGT and T2DM subjects and insulin sensitivity improved in both groups (358 ± 31.5 to 406 ; 263 ± 33 to 308 ± 54 ml.min⁻¹.m⁻², NGT and T2DM $p < 0.0001$). PTH concentrations decreased in both groups (from 68.7 ± 27.9 to 57.7 ± 40.9 pg/ml in NGT and from 79.7 ± 31.5 to 58.9 ± 27.2 pg/ml, $p < 0.001$), while 25OH-D increased in both groups (from 16.4 ± 13.1 to 22.0 ± 12.9 in NGT and from 13.2 ± 9.4 to 21.2 ± 14.9 ng/ml, $p < 0.002$).

Conclusion: In morbidly obese patients, high circulating concentrations of PTH and low vitamin D levels are associated with the presence of hyperglycaemia regardless of insulin sensitivity. Restrictive bariatric surgery rapidly corrects both vitamin D and PTH.

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Diabetes is the main factor accounting for hypomagnesaemia in obese subjects

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Background and aims: To determine whether the presence of type 2 diabetes and the degree of metabolic control are related to low serum magnesium levels in obese individuals.

Material and methods: A) Case-control study: magnesium was measured in 200 obese subjects (50 with type 2 diabetes [cases] and 150 without diabetes [controls]) prospectively recruited. B) Interventional study: the effect of bariatric surgery on serum magnesium levels was examined in a subset of 120 obese subjects (40 with type 2 diabetes and 80 without diabetes). Statistics: comparisons between groups were performed using Student *t* test for continuous variables and the χ^2 test for categorical variables. For the analysis of the interventional study, the Student-*t* test for paired data was used. The relationship between continuous variables was examined by the Pearson linear correlation test. A stepwise multiple linear regression analyses was performed in order to explore the variables independently related to serum magnesium. The variables included were fasting glucose, HbA1c, BMI, age, gender, and HOMA-IR. Because of collinearity between fasting blood glucose and HbA1c, two regression models were performed, which took into account each of these variables. All *p* values were based on a two-sided test of statistical significance. This study was approved by the Local Ethics Committee and was performed in accordance with the ethical standards laid down in the Helsinki Declaration (see World Medical Association: www.wma.net).

Results: Type 2 diabetic patients showed lower serum magnesium levels [0.75 ± 0.07 vs. 0.81 ± 0.06 mmol/l; mean difference -0.06 (95% CI -0.09 to -0.04); $p < 0.001$] than non-diabetic patients. Ten percent of the diabetic subjects, but none of the non-diabetic subjects, showed a serum magnesium concentration lower than 0.65 mmol/l. Significant negative correlations between magnesium and fasting glucose ($r = -0.443$; $p < 0.001$), HbA1c ($r = -0.460$; $p < 0.001$), HOMA-IR (log) ($r = -0.182$; $p = 0.015$) and BMI ($r = -0.142$; $p = 0.046$) were detected. Multiple linear regression analyses showed that both fasting glucose and HbA1c were independently related to serum magnesium. After bariatric surgery, serum magnesium increased only in those patients in whom diabetes had been resolved, but otherwise remained unchanged, with no difference in weight loss between the groups. Changes in serum magnesium negatively correlated with changes in fasting glucose and HbA1c. Baseline fasting glucose and HbA1c independently predicted magnesium changes in the multiple linear regression analysis.

Conclusions: Our results provide evidence that the presence of diabetes and the degree of metabolic control are essential in accounting for the lower levels of magnesium that exist in obese subjects. Since low serum magnesium levels are an independent cardiovascular risk factor and favour diabetic complications, the normalization of serum magnesium levels is another good reason for recommending the optimization of blood glucose in type 2 diabetic patients.

PS 036 Insulin: metabolites and actions

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Characteristics of insulin glargine signalling in rats

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Background and aims: Insulin glargine (GLA) is a long-acting insulin analogue that demonstrates safe and effective 24-hour glycemic control. In vitro, GLA has an insulin receptor (IR) profile similar to that of human insulin (HI) but has a slightly higher affinity for the insulin-like growth factor-1 receptor (IGF1R). [AspB10]Insulin (AspB10), the only insulin analog with proven carcinogenic activity, has IGF1R activity greater than HI and a higher IR affinity with a prolonged IR occupancy time when compared to GLA. In this study, we analyzed the time action profile of GLA in different tissues of rats with respect to pharmacological and signalling parameters and compared it to HI, insulin detemir (DET) and AspB10.

Materials and methods: Male Wistar rats were injected subcutaneously with 1 IU/kg of HI, GLA, DET or AspB10, and the effects on blood glucose and phosphorylation status of IR, IGF1R, AKT and ERK1/2 in tissue samples derived from muscle, fat, liver, heart and kidney investigated over time.

Results: After injection of HI and AspB10, glucose levels started to drop immediately and reached their minimum after 1 hour. Glucose lowering after injection of GLA reached the same minimum, however the onset of hypoglycemic action was delayed. Injection of an equal dose of DET failed to lower blood glucose in this setting and therefore was not further characterized. GLA treatment resulted in phosphorylation levels of IR and AKT that were comparable to HI although delayed in some tissues. In contrast, AspB10 treatment resulted in at least 2-3 fold higher phosphorylation levels and significantly longer duration of IR and AKT phosphorylation in most tissues in vivo, confirming the in vitro observations of increased affinity and occupancy time of the IR. Importantly, neither HI nor GLA nor AspB10 treatment resulted in any detectable IGF1R phosphorylation in muscle and heart tissue, whereas injection of IGF-1 increased the phosphorylation of this receptor.

Conclusion: The time course of insulin receptor signaling by human insulin and insulin glargine reflects the time course of their pharmacodynamic effects in a comparable and correlative way, which is distinctively different from [AspB10]Insulin.

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Analysis of signalling pathways stimulated by insulin, [Asp^{B10}]insulin and insulin glargine and its metabolites in the human mammary epithelial cell lines MCF7 and MCF10

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Background and aims: Insulin glargine (GLA) is a long-acting insulin analogue that in vitro displays a similar affinity for the insulin receptor (IR) as human insulin (HI) and an increased affinity for the insulin-like growth factor-1 receptor (IGF1R) comparable to that of [AspB10]insulin (AspB10). However, in contrast to AspB10 that is the only insulin analogue with proven tumorigenic properties in rats, GLA did not show increased incidence of mammary tumors in mice and rats in 2-year carcinogenicity studies. In both humans and animals, GLA is rapidly and significantly metabolized into the metabolites M1 and M2 that have a metabolic profile like GLA but a mitogenic profile identical to that of HI. The aim of this study was to compare the mitogenic activity and signaling behavior of GLA, M1 and M2 to IGF-1, HI and AspB10 in the human mammary epithelial cell lines MCF7 and MCF10a. These cell lines have different IGF1R/IR and IR-A/IR-B ratios (MCF7: 3.7:1 and 5:1; MCF10a 1.5:1 and 14:1) as measured by RT-PCR.

Materials and methods: MCF cells were incubated with increasing concentrations of HI, IGF-1, GLA or AspB10 and the amount of phosphorylation of AKT and ERK1/2 determined using western blotting. Thymidine incorporation into DNA was also determined.

Results: Compared to IGF-1, AspB10 and GLA showed a ~3- and ~2-fold lower EC₅₀ for AKT and ERK1/2 phosphorylation, respectively, and a more potent stimulation of thymidine incorporation in MCF7 cells. In contrast, the signaling behavior and mitogenicity of M1 and M2 were reduced com-

pared to HI. In MCF10a cells, AspB10 displayed increased mitogenic activity compared to HI that can be explained by its increased affinity and occupancy time at the IR, whereas GLA and its metabolites were comparable to HI.

Conclusion: Together the data indicate that in vitro mitogenicity of GLA cannot be directly linked to enhanced IGF1R affinity. In addition, in vitro data generated under artificial cell culture conditions cannot be translated into the in vivo situation, since biotransformation, distribution and the competition with levels of free endogenous IGF-1 critically affect the metabolic and mitogenic signaling properties of GLA in vivo as well. Only in vivo experiments generate evidence and explain the established efficacy and safety of GLA as observed in clinical practice and in long-term clinical trials. The current results emphasize the importance of studying the multiple steps in the action of an insulin analogue both in vitro and in vivo.

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Plasma exposure to insulin glargine and its metabolites in patients with type 1 diabetes

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Background and aims: After subcutaneous (SC) injection in vivo, glargine (GLA) is enzymatically transformed to metabolites M1 and M2 with loss of the di-arginines at position 30a–30b of the B chain but retention of the glycine for asparagine substitution at position A21 of the A chain. In vitro, GLA exhibits slightly higher affinity while M1 and M2 exhibit lower affinity for IGF-1 receptors than human insulin (HI). Due to technical constraints few data on the plasma concentration of GLA, M1, and M2 in humans exist.

Materials and methods: Plasma concentrations of GLA, M1, and M2 were determined from samples taken in a single-center, randomized, euglycemic glucose clamp study, where 12 male subjects with type 1 diabetes (BMI 25 kg/m²; A1C < 8.0%) per group received single SC doses of 0.3, 0.6, or 1.2 U/kg GLA. GLA, M1, and M2 were extracted using immunoaffinity columns and quantified by a specific liquid chromatography tandem mass spectrometry assay, without cross-reactivity to endogenous HI or other insulins. LLOQ was 200 pg/ml. The areas under the GLA, M1, and M2 curves (PK-AUC_{0–24}) were determined. The PD effect was determined from the AUC under the glucose infusion rate curve (PD-AUC_{0–24}).

Results: GLA and M2 were only detectable in approximately one third of patients and at a few time points only. When detectable, GLA and M2 exposure did not increase with increasing dose, and concentrations were far below endogenous interprandial plasma insulin concentrations of nondiabetic subjects. Plasma exposure of M1 increased with increasing dose; geometric mean (%CV) PK-AUC_{0–24} was 7 (61), 18 (75), and 23 (24) ng·h/ml at doses of 0.3, 0.6, and 1.2 U/kg, respectively. The geometric mean (%CV) PD-AUC_{0–24} was 522 (64), 2330 (37), and 5231 (28) mg/kg, respectively.

Conclusion: After subcutaneous injection of GLA in subjects with T1DM, there is a rapid removal of di-arginines with nearly total transformation of GLA into M1, accounting almost totally for the PD effect of injected GLA. In vivo exposure to GLA, if any, appears to be marginal. The results demonstrate that in vivo glargine is rapidly metabolized to active products that have lower affinity for IGF-1 than endogenous human insulin.

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Restoring metabolic control in diabetic rats by insulin replacement

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Background and aims: With current insulin therapy the physiological release to the portal vein is not reproduced since the subcutaneous (sc) route results in higher peripheral insulinization relative to the splanchnic region. This may hamper the optimal metabolic regulation in insulin-treated diabetics. We tested whether intraperitoneal (ip) insulin delivery could improve the performance of diabetic rats in regards to hepatic-specific insulin metabolic actions, namely glycogen and lipid synthesis.

Materials and methods: Male Wistar rats were induced diabetes by streptozotocin (65 mg/kg) injection in the ip cavity (glycemia: 534±22 mg/dL; plasma insulin: 0.5±0.2 µg/l), insulin replacement started 8 days afterwards and consisted of two daily injections (15 U/kg) via the sc (group I-SC) or ip (group I-IP) route for 10 days. In the last day of treatment rats (groups: C, non-diabetic controls; D, diabetics; I-SC and I-IP) were kept under deuterated water (²H₂O) overnight while feeding ad lib. and pathways to hepatic glycogen and lipid synthesis addressed by ²H NMR methods. Gene expression was quantified by qPCR.

Results: Hepatic glycogen (µmol/g of tissue dry weight) decreased with diabetes: 907±84, C; 320±51, D (P<0.05 vs C, I-SC and I-IP); and increased with insulin treatments: 680±52, I-SC (P<0.05 vs D and C); 814±63, I-IP (P<0.05 vs D). Indirect (gluconeogenic) pathway contribution to glycogen increased with diabetes: 54±4%, C; 95±3%, D (P<0.05 vs C, I-SC and I-IP); and was restored with insulin: 47±2%, I-SC (P<0.05 vs D) and 49±2%, I-IP (P<0.05 vs D). Hepatic triglycerides (HTG, µmol/g of tissue dry weight) increased with diabetes: 212±22, C; 312±20, D (P<0.05 vs C); and remain elevated with insulin treatments: 301±15, I-SC (P<0.05 vs C) and 287±11, I-IP (P<0.05 vs C). Contribution of de novo lipogenesis (DNL) to HTG was 16±2% in C, only 2±1% in D (P<0.05 vs C, I-SC and I-IP), but increased with insulin replacement: 7±1%, I-SC (P<0.05 vs D and C) and 8±1%, I-IP (P<0.05 vs D and C). Gene expression analysis is shown in Figure 1.

Conclusion: Both insulin replacement therapies restored the direct/indirect pathway contributions to glycogen synthesis but hepatic stores were only recovered to control values by the ip route. The expression of glycolytic and lipogenic enzymes was stimulated, consistent with enhance DNL as determined by the ²H₂O method. Neither therapy effectively restrained the expression of gluconeogenic enzymes and CPT1a. Hence, gluconeogenic G6P synthesis, fuelled by mitochondrial fat oxidation, was also likely to be less controlled.

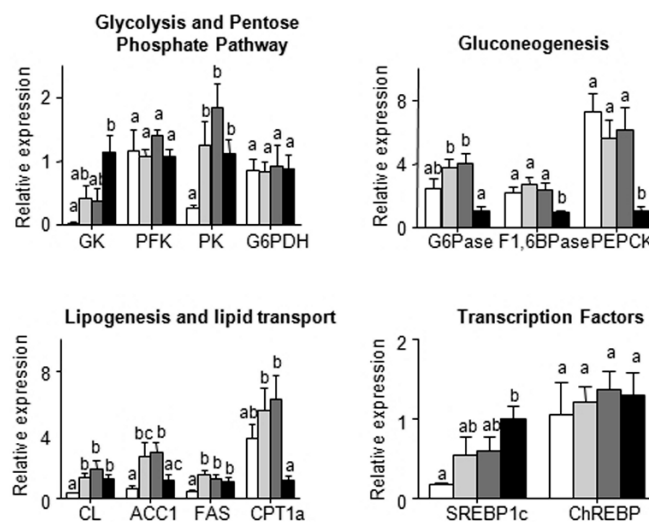


Figure 1: Gene expression determined in livers from groups D, white bars; I-SC, light grey bars; I-IP, dark grey bars and C, black bars; different letters indicate significant differences (P<0.05).

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Effect of insulin detemir on adipocyte lipid metabolism in patients with type 2 diabetes

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Background and aims: Subcutaneous administration of insulin may cause peripheral hyperinsulinaemia leading to abnormalities in adipocyte metabolism and possible weight gain. Insulin detemir has been shown to cause less weight gain than other insulin treatments. This may be related to insulin detemir's hepatoselective action which has the potential to restore the physiological insulin gradient.

Materials and methods: In a single-centre, 24 week, randomized parallel-group trial the effect of insulin detemir on adipocyte lipid metabolism was investigated in 22 type 2 diabetic subjects (14M, 8F, aged 60.4± 8.1y), BMI 32.3

$\pm 3.6 \text{ kg/m}^2$) (means \pm SD). Patients were optimized for 8 weeks with NPH insulin and then randomized to receive either insulin detemir or NPH insulin for 16 weeks. Weight change, glycemic control, NEFA and triglycerides were measured after 8 weeks and after 16-week treatment period. In a sub-set, basal and stimulated lipolytic activity, lipoprotein lipase (LPL) mass and activity were measured in subcutaneous fat tissue biopsy at the same time points.

Results: Weight change over the treatment period was $-0.62 \pm 0.84 \text{ kg}$ with insulin detemir and $+2.19 \pm 1.03 \text{ kg}$ with NPH ($p=0.049$). There was no difference in HbA1c. Fasting non-esterified fatty acids (NEFA) decreased significantly with detemir compared to NPH ($p=0.02$). Basal lipolysis in the fat biopsies also showed a significant reduction with detemir compared to NPH over the treatment period ($p<0.04$). LPL mass and activity increased with insulin detemir compared to NPH ($p=0.005$, $p=0.006$).

Conclusion: The decrease in basal NEFA levels and basal lipolysis and increase in LPL mass and activity suggests an improvement in fasting insulin sensitivity which may be due to a greater hepatoselective action of detemir reducing overinsulinisation of adipose tissue or may be the consequence of improved insulin sensitivity due to weight loss.

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Differential roles of MEK/ERK1/2 in human insulin- and insulin glargine-induced proliferation of T24 and HepG2 cells

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Background and aims: MEK/ERK1/2 has been implicated in the mitogenic effects of insulin and its analogues in malignant cells. The results are varied with different experimental settings. This study was to compare the effects of MEK/ERK1/2 pathway in human insulin- and insulin glargine-induced proliferation in human bladder cancer T24 and human hepatocellular carcinoma HepG2 cell lines.

Materials and methods: Cultured T24 or HepG2 cells were treated with a selective MEK or ERK1/2 inhibitor either alone or in combination with insulin or glargine at different doses for the indicated time courses. U0126 is a highly selective inhibitor of both MEK1 and MEK2. The ERK inhibitor, (3-(2-Aminoethyl)-5-((4-ethoxy-phenyl)methylene)-2,4-thiazolidinedione), is known to block ERK-mediated phosphorylation of ribosomal S6 kinase-1 (RSK-1) and ternary complex factor Elk-1. Cell proliferation was evaluated by CCK-8 assay. Protein expression of insulin receptor (IR), type 1 insulin-like growth factor receptor (IGF1R), phosphorylated ERK1/2 (pERK1/2) and total ERK1/2 were detected by Western blotting.

Results: Human insulin and insulin glargine similarly promoted proliferation of HepG2 and T24 cells at 10 IU/L and 100IU/L. IR and IGF1R were detected in both cell lines. In T24 cells, insulin and glargine induced increases in pERK1/2 level at 15min, 30min and 60min while no change was detected at 24h. By contrast, pERK1/2 protein was abundantly expressed in untreated HepG2 cells. No change in pERK1/2 level was observed at early or late time points with either insulin or glargine treatment in HepG2 cells. Basal T24 and HepG2 cell proliferation was reduced in association with decreased pERK1/2 level by U0126. Insulin- and glargine-induced proliferation was blocked by U0126 and the ERK inhibitor in HepG2 cells, while unaffected in T24 cells.

Conclusion: Human insulin and insulin glargine-induced proliferation is MEK/ERK1/2 dependent in HepG2, not in T24 cells. The effect of MEK/ERK1/2 in the mitogenicity of insulin or its analogues may be cell lineage specific.

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Differential metabolic and mitogenic signalling of Asp^{B10} and insulin glargine *in vitro* and *in vivo*

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Background and aims: Treatment of diabetic patients with insulin analogues has been shown to provide a more efficient, reproducible, and convenient therapy than regular insulin. The analogues may vary from insulin with respect to metabolic potency, stability or onset and duration of action

that is achieved by either sequence or secondary structural modifications. These changes may lead to an altered activation profile of insulin (IR) and/or insulin-like growth factor-1 (IGF1R) receptor signaling pathways and may change metabolic or mitogenic responses. [Asp^{B10}]Insulin (AspB10) is an insulin analogue that was withdrawn from clinical development due to a higher incidence of breast cancer in rats. *In vitro*, AspB10 displays higher affinity toward both IR and IGF1R, a prolonged occupancy time at the IR and a higher proliferation rate in mammalian cell lines. This has led to the general belief that insulin analogues with increased affinity for IGF1R *in vitro* have increased growth promoting activity *in vivo* as well. Insulin glargine (GLA) has an *in vitro* IR signaling and metabolic profile comparable to that of insulin (HI) and displays slightly greater affinity for IGF1R. This long-acting analogue undergoes rapid and significant metabolism in humans and animals, leading to early formation of two main metabolites (M1 and M2) that have *in vitro* metabolic and mitogenic profiles comparable with HI. Our aim was to investigate *in vivo* the metabolic and mitogenic signaling profile of GLA and AspB10 compared with HI.

Materials and methods: Male Wistar rats were injected with 1 or 12.5 U/kg subcutaneously of HI, GLA or AspB10 or with 1 mg/kg des1-3 IGF-1 intravenously. Samples of skeletal muscle, liver, adipose tissue, kidney and heart were harvested at various times for analysis of IR, IGF1R, AKT and ERK1/2 phosphorylation.

Results: Injection of neither HI nor AspB10 nor GLA induced IGF1R autophosphorylation in responsive tissues, whereas injection of IGF-1 produced a robust activation of the receptor. Injection of AspB10 induced an increased and prolonged phosphorylation of IR signaling molecules in several tissues. This IR signaling pattern of AspB10 *in vivo* was distinctly different from that of HI and GLA and confirms earlier *in vitro* findings.

Conclusion: We hypothesize that the carcinogenic effect of AspB10 is based on its altered IR activation profile and is independent of its slightly greater IGF1R affinity *in vitro*.

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Impairment of insulin clearance rather than beta cell function in the progression to type 2 diabetes

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Background and aims: From the insulin resistance state to the progression to type 2 diabetes the major metabolic dysfunctions are associated to β -cell secretion. The consensual overview is that, first β -cell increases insulin secretion (hyperinsulinemia) in order to compensate the burst of high glucose levels, and after this overproduction, β -cell drop its capacity to produce insulin, culminating in type 2 diabetes. Association between plasma insulin levels and β -cell secretion are commonly used. However, the evaluation of C-peptide seems to be a better predictor of β -cell function rather than insulin, and that is due to several alterations on insulin clearance that seems to occur during the progression of the disease. Therefore, our hypothesis is that from healthy to insulin resistance states, the major complications observed are not due to β -cell insulin/C-peptide secretion, but mainly due to a decrease on insulin clearance.

Materials and methods: Male Wistar rats (12 weeks old, control vs high-sucrose diet) were used. The high-sucrose diet group consisted in 2 groups of animals: Suc 4 - fed with a high-sucrose diet (35% (w/v)) for a 4 week period (8-12 weeks of age); Suc 9 - fed with a high-sucrose diet (35% (w/v)) for a 9 week period (3-12 weeks of age). Insulin sensitivity was assessed by the Rapid Insulin Sensitivity Test (RIST), in the fed state. To evaluate glucose excursions, insulin and C-peptide levels, a mixed meal (BOOST[®]) was administered and a Meal Tolerance Test (MTT) was performed. Blood samples were taken at time 2, 5, 10, 20, 30, 45, 60, 90 and 120 minutes after the BOOST[®]. Insulin clearance was calculated by the ratio of the area under the curve (AUC) of C-peptide and AUC of insulin.

Results: Postprandial insulin action was determined in both groups and we observed that high-sucrose diet animals showed a decreased in insulin action, which was not aggravated with the duration of the diet (Control: 193.2 ± 11.2 , n=9, Suc 4: 99.7 ± 7.2 , n=4, Suc 9: $107.4 \pm 14.9 \text{ mg glucose/kg bw}$, $p<0.001$). Only the Suc 9 group was hyperglycemic, both in the fasted and fed state, in comparison to the control group (Fasting: Control - 76.1 ± 3.4 , n=9, Suc 4 - 75.8 ± 3.4 , n=4, Suc 9 - $100.9 \pm 5.7 \text{ mg/dl}$, $p<0.05$, n=9; Fed: Control - 106.6 ± 3.0 , n=9, Suc 4 - 105.08 ± 6.7 , n=4, Suc 9 - $127.1 \pm 6.3 \text{ mg/dl}$, $p<0.05$, n=9). The results obtained during the MTT showed that there was only an increase in glucose excursions in the Suc 9 animals (AUC: Control - 14733 ± 445.6 ,

n=9, Suc 4 - 14660 ± 951.9 , n=4, Suc 9 - 16976 ± 1238 mg/dl, n=9). The evaluation of insulin clearance showed that Suc 4 and Suc 9 animals had a 34.1% and a 56.6% reduction on insulin clearance, respectively. From the 4 weeks to the 9 weeks of high-sucrose diet, the major alterations observed were the plasma insulin levels, while the C-peptide levels and insulin action remained unchanged, which indicates that it is the insulin clearance that is affecting the progression of the disease.

Conclusion: The results presented herein allow us to conclude that both Suc 4 and Suc 9 animals are insulin resistant with a decrease on insulin action that is not aggravated with the diet exposition. Comparing both groups of high-sucrose diet animals we observed that C-peptide levels are not altered between the groups but the major alterations are occurring on plasma insulin levels. The results obtained herein showed that from pre-diabetes to diabetes the major alteration that are occurring are due to dysfunctions on insulin clearance rather than β -cell dysfunction.

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Reappraisal of hyperbolic relationship between insulin sensitivity (Si) and insulin secretion (β)

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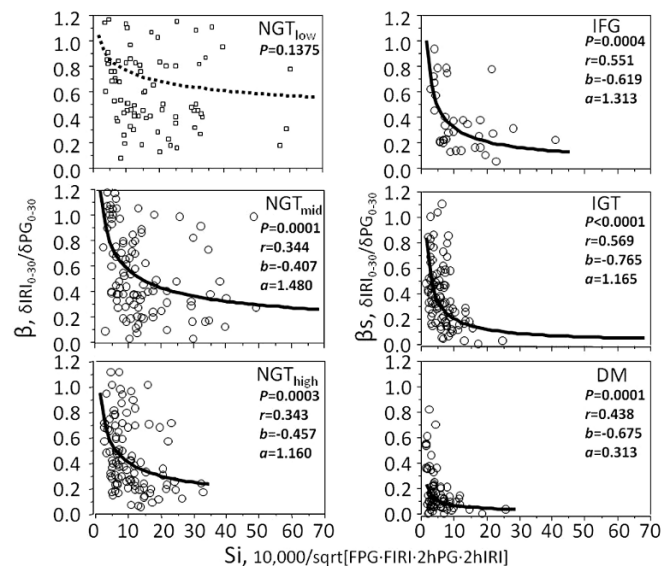
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Background and aims: In healthy subjects, increase or decrease of Si is considered followed by lowered or elevated β , respectively, to maintain euglycaemia. Hyperbolic correlation between Si and β is in support of such idea, for which glucose allostasis has been proposed as an underlying mechanism especially in subjects with normal glucose tolerance (NGT). We systematically reexamined the issue.

Materials and methods: We analyzed data from 533 health examinees (the mean age 52.8 yrs and body mass index 23.6 kg/m^2). Those with a history of diabetes mellitus (DM) were excluded. Plasma glucose (PG) and immuno-reactive insulin (IRI) were determined in 0, 30 and 120 min samples at 75 g OGTT. In Analysis I (A_I), the participants were classified according to the current diagnostic criteria as NGT (n=328), impaired fasting glycaemia (IFG) (n=37), impaired glucose tolerance (IGT) (n=96) and DM (n=72). NGT was subdivided into NGT_{low} (n=103), NGT_{mid} (n=118) and NGT_{high} (n=107) by fasting PG (FPG) tertile, ≤ 4.9 , 5.0–5.4 and 5.5–6.0 mmol/l. In Analysis II (A_{II}), the subjects were classified based on FPG sextile. FPG range was ≤ 4.8 , 4.9–5.1, 5.2–5.4, 5.5–5.7, 5.8–6.3 and ≥ 6.4 mmol/l, for S_1 (n=91), S_2 (n=84), S_3 (n=83), S_4 (n=96), S_5 (n=94) and S_6 (n=85), respectively. As an index of β , $\delta IRI_{0-30}/\delta PG_{0-30}$ at 75 g OGTT was used. As a measure of Si, an index proposed by Matsuda, $Si = 10,000/\sqrt{[FPG \cdot FIRI \cdot 2hPG \cdot 2hIRI]}$, was used, where FIRI, 2hPG and 2hIRI are fasting IRI, 2-h post glucose PG and IRI at 75 g OGTT, respectively. Hyperbolic regression of β to Si, $[\beta] = a \cdot [Si]^b$, was compared between the 6 groups in A_I and A_{II} , respectively. Correlation coefficient (r) was obtained after log transformation by ordinary least-squares regression. Disposition index (DI) and beta cell demand index (BCDI) were calculated assuming each group of subjects is on a different hyperbola, i.e., $[Si]^b$ not $[Si]^{-1}$ was used.

Results: In A_I , Si- β correlation was most strong and hyperbolic in IGT, progressively less so in the groups of subjects with better glucose tolerance, and absent in NGT_{low} (Figure). b was significantly different from -1 in all NGT groups ($P < 0.01$ for all). The regression line was progressively nearer to the origin from NGT_{mid} to DM. The best-fit line for $[\beta] = a \cdot [Si]^b$ based on all values in each group is shown, with the same axes ($\beta = 0 \sim 1.2$ and $Si = 0 \sim 70$ with metric unit) for all groups. Results were qualitatively similar in A_{II} . Namely, Si- β correlation was most strong and hyperbolic in S_5 , progressively less so in the groups of subjects with better glucose tolerance, and absent in S_2 and S_1 . b was significantly different from -1 in all groups. The mean DI was significantly smaller in DM and S_6 than other groups in A_I and A_{II} , respectively ($P < 0.01$ for both). BCDI positively correlated with 2hPG in 3 NGT groups in A_I and in all sextiles in A_{II} ($P < 0.01$ for all), but not with FPG in any group.

Conclusion: Si- β correlation, which is not exactly hyperbolic, fades away in the group of subjects with superb glucose regulation, and glucose allostasis was confirmed on the basis of 2hPG.



PS 037 Vascular functions and metabolic actions

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Glucagon-like peptide-1 protects against tert-Butyl hydroperoxide-induced HUVEC apoptosis: involvement of PI3k/Akt pathway

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Background and aims: Endothelial cells have a robust capacity to recover vascular injury and maintain intimal layer integrity. Endothelial apoptosis is involved in the formation of atherosclerosis. In this study we investigated whether the anti-diabetic hormone exendin-4 could prevent oxidative stress-induced human umbilical vein endothelial cells (HUVECs) apoptosis and its underlying mechanisms.

Materials and methods: HUVECs were cultured in vitro and exposed to tert-butyl hydroperoxide (t-BHP), an H_2O_2 analogue, or t-BHP plus Exendin-4 (2.5, 5, 12.5 or 25nmol/l). Cell viability was determined by MTT assay and apoptosis was detected by fluorescence microscopic analysis after Hoechst33342/PI staining. HUVECs were pre-incubated with exendin-4 (25nmol/l) for 18 h, then phosphatidylinositol 3-kinase (PI3k) inhibitor, wortmannin (100nmol/l) were added to the medium 30 min before t-BHP exposure. The protein expression of phosphorylation of Akt, Bcl-2 and caspase-3 were measured by Western blot.

Results: The results showed that oxidative stress-induced endothelial cells injury was partially mediated by endothelial cell apoptosis. After pre-treatment with exendin-4 (25nmol/l), cell viability increased by 46.50% ($P<0.05$), and the number of apoptosis, characterized by chromatin condensation and marginalization, decreased significantly (7.98 ± 1.45 vs 36.00 ± 1.25 , $P<0.05$), compared with the t-BHP treated group alone. Going a further step, we examined the underlying regulation. Pre-incubation of HUVECs with exendin-4 resulted a reduction of active caspase-3 (by 48.53%, $P<0.05$), an enhancement of phosphorylated Akt (by 1.78 times, $P<0.05$) and an increase in anti-apoptotic protein Bcl-2 expression (by 1.08 times, $P<0.05$), compared with the t-BHP group. These results indicate that exendin-4 can protect t-BHP-induced HUVECs apoptosis. However, this protective effect was inhibited by the phosphatidylinositol-3 kinase (PI3k) inhibitor wortmannin.

Conclusion: Our findings showed that GLP-1 analogue exendin-4 has a protective effect against oxidative stress-induced endothelial injury by inhibiting cell apoptosis, which may associate to the regulation of survival pathway PI3k/Akt and anti-apoptosis protein Bcl-2.

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Insulin sensitivity and paraoxonase-1 gene polymorphisms independently affect endothelial function in non-diabetic males with essential hypertension

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Background and aims: Paraoxonase-1 (PON-1), a high-density lipoprotein-bound esterase, exerts a powerful anti-oxidant and anti-inflammatory effect. Variants of PON-1 gene have been shown to impair these properties. In the present study we have tested whether common PON-1 genetic variants may contribute to impaired endothelial function of non-diabetic hypertensive patients independently of haemodynamic and metabolic features.

Patients and methods: The Q192R and L55M variants of PON-1 gene were determined in 95 drug-naïve non-diabetic hypertensive male subjects (age 53 ± 13 years; BMI 26.4 ± 2.9 kg/m²; BP $146\pm15/92\pm10$ mmHg). Endothelial function was assessed by forearm blood flow (FBF) response to intra-brachial acetylcholine (Ach) and sodium nitroprussiate (SNP) infusion. In all subjects 24-hr ambulatory blood pressure was recorded. Finally, lipid profile, hs-CRP and insulin sensitivity (OGTT-based ISI) were determined.

Results: Genotype frequencies for Q192R were 56.8% QQ, 37.9% QR, and 5.3% RR; for L55M were 50.5% LL, 35.8% LM, and 13.7% MM, both consistent with HWE. There were no differences in any of the clinical and biochemical parameters according to alleles distributions for both PON-1 genetic variants. Similarly, there was no difference in basal FBF. The presence of the Q allele was not associated with a significant difference in endothelial response

to Ach or SNP. In contrast the presence of the M allele was associated with impaired FBF response to Ach ($AUC_{Ach-FBF}$ LL: 234 ± 106 ; LM: 187 ± 75 , and MM: 155 ± 58 ml/min*100ml tissue*15min; $p=0.009$). Marginally significant changes in SPN response were also found ($AUC_{SNP-FBF}$ LL: 198 ± 61 , LM: 171 ± 43 , and MM: 177 ± 42 ml/min*100ml tissue*15min; $p=0.057$). On multiple stepwise regression analysis, no parameter (age, BMI, fasting and 2-hr OGTT plasma glucose, HbA1c, hs-CRP, fasting plasma insulin, triglycerides, total-, LDL-, HDL-cholesterol) was associated with $AUC_{Ach-FBF}$, while ISI in a positive manner ($r=0.372$, $p=0.001$) and M allele in a negative one (cumulative $r=0.452$, $p=0.007$) remained independently associated with endothelial function. A weaker, although statistically significant, effect of the M allele was found to predict also $AUC_{SNP-FBF}$ ($p=0.027$), independently of OGTT-glucose- AUC (0.008) and BMI ($p=0.029$).

Conclusions: Insulin resistance and the M allele of the PON-1 L55M polymorphism, but not the Q192R ones are independently associated with impaired endothelial responses to acetylcholine and, to a lesser extent, nitroprussiate. Therefore, the coexistence of these two conditions may represent a worsening effect on the cardiovascular risk of hypertensive subjects.

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Methylglyoxal impairs insulin signalling and endothelial function both in vitro and in vivo

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Background and aims: It has now become evident that insulin exerts a direct action on vascular cells, thereby conditioning the outcome and progression of vascular complication associated with diabetes. However, the mechanisms through which insulin signaling is impaired in the vascular endothelium remain still unclear. Chronic hyperglycaemia per se promotes insulin resistance and plays a pivotal role in the outcome and progression of diabetes-associated vascular complications. Hyperglycaemia may act through different mechanisms, including generation of advanced glycation end products (AGEs). In this work we evaluated the role of the AGEs precursor methylglyoxal (MG) in the generation of endothelial insulin-resistance in cellular and animal models.

Materials and methods: Time-courses experiments were performed on NIH-3T3 fibroblasts and bovine aortic endothelial cells (BAEC) incubated with different concentrations of MG. The glyoxalase1 inhibitor SpBrBzGSHCp2 was used to increase the endogenous levels of MG. For the in vivo study, C57BL6 mice were intraperitoneally injected with a MG solution at steadily increasing concentrations (50 to 75mg/kg) for 7 weeks.

Results: MG incubation induced a 50% reduction of IRS1 phosphorylation, the loss of IRS1-p85 interaction and of the downstream Akt activation in response to insulin, whilst MAPK was more active in both NIH3T3 and BAEC treated with MG. Furthermore, MG was able to inhibit insulin-induced eNOS activation in BAEC, as shown by the decrease of Ser1177eNOS phosphorylation by 3.5-fold together with the increased phosphorylation at the inhibitory Thr495 site of eNOS by 2-fold, as compared to control cells. This was paralleled by a 60% decrease of insulin-induced NO production in BAEC. Similar results were obtained in BAEC treated with the glyoxalase1 inhibitor SpBrBzGSHCp2 compared to control BAEC. Intraperitoneally administration of MG to mice caused insulin resistance (ITT AUC: C57MG 10163 ± 1979 vs C57 7787 ± 1174 mg/dl/120', $p=0.01$) and reduced serum NO by 2.5-fold compared to untreated mice. Western blots of lysates of aortae from MG-treated mice revealed a reduction of insulin-induced Akt activation.

Conclusion: These results suggest that MG impairs insulin signaling in endothelial cells and insulin effect on endothelial NO production both in vitro and in vivo. Understanding the molecular mechanisms by which hyperglycaemia compromises insulin action in vascular cells may allow to develop new strategies to preserve endothelial function in diabetic subjects.

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Cardiometabolic side effects of glucocorticoid treatment in humans: role of impaired microvascular function

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Background and aims: Glucocorticoid (GC) treatment induces several cardiometabolic side effects including insulin resistance, glucose intolerance and hypertension. The mechanisms underlying these side effects are presently largely unknown. Microvascular dysfunction, characterised by impaired capillary recruitment, affects both flow resistance and perfusion, and may contribute to insulin resistance and hypertension, as was shown in obese and diabetic populations. The objective of this study was to assess the role of microvascular dysfunction in GC-induced cardiometabolic side effects.

Materials and methods: We conducted a double-blind randomised placebo-controlled dose-response trial in which 32 healthy normoglycaemic men (age: 21.0 ± 2.1 years, BMI: 21.9 ± 1.7 kg m⁻²) were treated with either prednisolone (PRED) 30 mg q.d. (PRED30), PRED 7.5 mg q.d. (PRED7.5) or placebo (PLB) for two weeks. At baseline and at day 14 of treatment, a hyperinsulinaemic-euglycaemic clamp at 40 mU m⁻² min⁻¹ was performed to measure insulin sensitivity. On the same days, finger nail fold capillaroscopy was performed to assess baseline capillary density and capillary recruitment following arterial occlusion, both in the fasted state and during hyperinsulinaemia. Moreover, blood pressure was recorded before and during treatment.

Results: PRED treatment dose-dependently decreased insulin sensitivity vs. PLB: mean differences: -2.1 ± 0.8 mg kg⁻¹ min⁻¹ ($P=0.021$) for PRED7.5 and -4.5 ± 0.7 mg kg⁻¹ min⁻¹ ($P<0.001$) for PRED30. Capillary recruitment in the fasted state was not affected by PRED7.5 ($P=0.785$) or PRED30 ($P=0.235$) as compared to PLB. Insulin infusion stimulated capillary recruitment by $13 \pm 5\%$ ($P<0.001$) compared to the fasted state, an effect that was non-significantly reduced by PRED7.5 ($P=0.064$ vs. PLB) and abolished by PRED30 ($P<0.001$ vs. PLB). Systolic blood pressure (SBP) was non-significantly augmented by PRED7.5 (2 ± 1.8 mmHg; $P=0.347$), but increased significantly by 6 ± 1.7 mmHg ($P=0.002$) during PRED30 treatment. The treatment-induced decline in insulin-stimulated capillary recruitment was correlated to both changes in insulin sensitivity ($\beta=-0.763$; $P<0.001$) and SBP ($\beta=0.542$; $P=0.002$).

Conclusion: Microvascular function was dose-dependently impaired in healthy men receiving 14 days of GC-treatment, shown by impaired insulin-induced capillary recruitment. Microvascular dysfunction may be involved in a number of GC-induced cardiometabolic side effects, including insulin resistance and hypertension.

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iNOS mediates S-nitrosylation of IR, IRS-1 and Akt and insulin resistance in the skeletal muscle of aging rodents

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Background and aims: It is increasingly recognized that protein S-nitrosylation, the addition of a nitric oxide group to cysteine thiols, serves an important role in a wide range of signaling pathways. Accumulating number of evidences demonstrate that exogenous nitric oxide (NO) and the NO produced by inducible nitric oxide synthase (iNOS) can induce S-nitrosylation of proteins involved in the early steps of insulin signaling pathway inducing insulin resistance. The aim of the present study was to examine iNOS expression, the S-nitrosylation of proteins involved in insulin signaling pathway and insulin sensitivity in young and old rodents.

Materials and methods: Pharmacological (L-NIL; 80 mg/kg body weight), physiological (swimming exercise) and genetic (iNOS-null mice) approaches were combined with euglycaemic-hyperinsulinaemic clamp, Western blot and biotin-switch methods to investigate the insulin sensitivity and signaling in the skeletal muscle of young and old rodents.

Results: We found that aging increased iNOS expression and S-nitrosylation of insulin receptor (IR), insulin receptor substrate-1 (IRS-1) and Akt and reduced the insulin sensitivity in the skeletal muscle when compared to young rodents. However, aging-induced S-nitrosylation and insulin resistance were blunted in muscle of aged iNOS-null mice. Furthermore, the treatment with

pharmacological iNOS activity inhibitor, (L-N⁶-(iminoethyl)-lysine acetate (L-NIL) was able to reduce S-nitrosylation and increased the tyrosine phosphorylation of IR, IRS-1 and serine phosphorylation of Akt and restored insulin sensitivity in muscle of aged rodents. Interestingly, a single bout of exercise reduced iNOS expression and S-nitrosylation of IR, IRS-1 and Akt. In parallel, exercise increased the tyrosine phosphorylation of IR, IRS-1 and serine phosphorylation of Akt and restored insulin sensitivity in the muscle of old animals.

Conclusion: These results suggest that aging-associated S-nitrosylation of IR, IRS-1 and Akt mediated by the iNOS activity may be an important contributing factor to the insulin resistance.

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A double-blind, randomised trial to evaluate the effects of 300 mg Aliskiren compared to 5 mg Amlodipine on IR and endothelial dysfunction in hypertensive patients with metabolic syndrome

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Background and aims: This double blind, randomized, parallel study was designed to evaluate the effects of 300 mg Aliskiren (ALI) and 5 mg Amlodipine (AML) on endothelial function in patients (pts) with metabolic syndrome, to explore effects on insulin sensitivity, and to evaluate effects on peripheral arterial compliance/vascular stiffness.

Materials and methods: Main inclusion criteria: Male/female, 18-65y/o, Metabolic syndrome (ATP III criteria) and BMI < 42, Δ MBF $\leq 35\%$, mean Glucose INFusion (GINF) rate ≤ 6.5 mg/kg/min, Impaired Glucose Tolerance or Impaired Fasting glucose, SBP ≥ 130 mmHg and/or DBP ≥ 80 mmHg. Pts with SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg and with Diabetes were excluded. Endothelial function was measured by the change in myocardial blood flow (MBF) between rest and CPT, using PET scan, at baseline and End of Study (End). Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp at baseline and End. GINF was determined as the arithmetic mean of the glucose infusion rate during the last 30 minutes of the clamp. For Δ MBF and GINF changes from baseline were analyzed by a 2-sample t-test. For arterial compliance, changes from baseline to End for central systolic/diastolic BP and central mean pressure were measured. Oral glucose tolerance test (OGTT) was performed at baseline and End. Change from baseline in arterial compliance parameters, and time-matched change from baseline in OGTT results were subjected to a linear mixed effects model analysis with treatment, time, treatment by time interaction as fixed effects and subject nested within treatment as a random effect. Time-matched change from baseline at each time point was compared between the 2 treatment groups.

Results: 48 pts (all Caucasian Hispanic) were randomized to either 12 weeks treatment with ALI or AML. Both treatments were well tolerated; no deaths or Serious Adverse Events were reported. Two pts on AML progressed into diabetes mellitus during the study, while all pts on ALI remained within pre-diabetic limits. Aliskiren and AML both improved MBF and GINF; however, between treatment differences were not significant. In contrast, OGTT showed an increase in insulin after ALI treatment, whereas a reduction in insulin was observed after AML treatment. No changes in glucose concentrations were observed. Reductions in central systolic pressure, central diastolic pressure, and central mean pressure were statistically significantly greater with ALI treatment, while peripheral BP did not show a difference between treatments. Reductions in UACR and endothelin-1 with ALI were also statistically significant from baseline.

Conclusion: Both ALI and AML improved endothelial function and insulin sensitivity, with no significant difference observed between treatments. Urinary albumin and endothelin-1, as well as central systolic pressure, central diastolic pressure, and central mean pressure improved significantly after ALI treatment. Two pts on AML but none on ALI pts progressed to overt diabetes. Increased OGTT insulin concentrations with ALI suggest that ALI may improve B-cell function in pre-diabetes. However, as the study was not designed to evaluate B-cell function, this mechanism merits future exploration.

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Effects of systemic nitric oxide blockade on insulin secretion and action

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Background and aims: In isolated β -cells, nitric oxide (NO) is synthesised in response to glucose and exerts a negative feedback on insulin release. Preliminary *in vitro* data suggest that NO downregulates insulin-degrading enzyme activity, while peripheral NO release from endothelial cells in man has been suggested to favour insulin action. We designed this study to test whether in humans systemic inhibition of endogenous NO synthesis affects insulin secretion and/or insulin action.

Material and methods: Three groups of non-diabetic subjects (n=5 each) underwent two hyperglycaemic (+7 mmol/L) clamp studies, during which either saline or N-(G)-nitro-L-arginine methylester (L-NAME) were co-infused throughout the clamp at rates of 5, 10, and 20 $\mu\text{g min}^{-1}\text{kg}^{-1}$. First phase (1stP), second phase (2ndP), and arginine-induced (ARG) insulin secretion were evaluated from the areas under the plasma C-peptide (AUC-Cp) and insulin (AUC-Ins) concentration curves. Insulin action was evaluated as mean glucose infusion rate of the last 40 min of the clamp either as the M value or as M normalised by the steady-state plasma insulin concentration (M/I).

Results: While saline had no effect on mean blood pressure or heart rate, L-NAME induced a significant, dose-dependent rise in the former (5 \pm 1, 8 \pm 1, and 20 \pm 2 mmHg, respectively, $p<0.01$ by ANOVA) and a drop in the latter (3 \pm 1, 5 \pm 1, and 12 \pm 2 b/min). When insulin secretion was evaluated as AUC-Cp, L-NAME did not affect any of the 3 responses. In contrast, when evaluated as AUC-Ins, L-NAME infusion resulted in a dose-dependent inhibition (as percent of the corresponding saline response) of 1stP (by 112 \pm 13, 85 \pm 6, and 47 \pm 8%, $p<0.05$), 2ndP (by 86 \pm 13, 79 \pm 13, and 48 \pm 13%, $p<0.05$) and ARG (by 102 \pm 11, 107 \pm 11, 61 \pm 9%, $p<0.05$ only for the highest L-NAME dose). Insulin action was impaired only at the highest L-NAME dose (M=54 \pm 8%, $p<0.01$), but this reduction was in proportion to the lower steady-state insulin levels achieved during L-NAME infusion (M/I= 125 \pm 26%, $p=\text{ns}$).

Conclusion: In man, systemic NO blockade does not affect either insulin secretion or peripheral insulin action but drastically increases plasma insulin removal. Whether the loss of this novel function of NO contributes to glucose intolerance remains to be determined.

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Liraglutide induced anorexia is not mediated by brainstem GLP-1 neurons

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Background and aims: The Glucagon-Like Peptide-1 (GLP-1) analogue liraglutide effectively lowers blood glucose as a treatment for type 2 diabetes. Liraglutide also reduces food intake and body weight in diabetes patients and in non-diabetic obese patients as well as in numerous animal models. Liraglutide is approved for the treatment of type 2 diabetes, and is in clinical development for the treatment of obesity. The native GLP-1 hormone has been shown to lower energy intake through a mechanism of increasing feelings of satiety and reducing feelings of hunger. However, the molecular mechanism for these effects are not fully understood and may have both peripheral and central components. Since GLP-1 is produced in the brainstem solitary tract nucleus it is of special interest to explore if those neurons are involved in mediating the effect of peripherally administered liraglutide.

Materials and methods: We have used the immediate early gene c-Fos to identify key appetite regulatory brain areas activated following liraglutide administration. To be able to fully discriminate between effects of drug (liraglutide, 0.1 mg/kg) and effects of feeding, rats were dosed before lights out in the presence (fed) or absence (unfed) of food. Vehicle groups were also included (veh). The study was terminated by perfusion fixation 4 hours later, brains were removed, cut into frozen sections and immunoreacted for c-Fos.

Results: Irrespective of feeding condition liraglutide increased c-Fos in the area postrema, the nucleus of the solitary tract, the lateral parabrachial nucleus and the central nucleus of amygdala, whereas liraglutide decreased the number of c-Fos positive nuclei in the hypothalamic arcuate nucleus. Given the role of brainstem GLP-1 neurons in appetite regulation we speculated that liraglutide induced anorexia could be mediated by activation of these neurons. Interestingly, double immunofluorescence revealed that liraglutide reduced the fraction of activated brainstem GLP-1 neurons when food was present (veh fed 54 \pm 7; lira fed 33 \pm 4%; $P<0.05$). However, in the absence of food none of the GLP-1 neurons expressed c-Fos (veh unfed 0.2 \pm 0.2; lira unfed 0.6 \pm 0.4%) indicating that brainstem GLP-1 neurons were activated by feeding only. Liraglutide administration reduced food intake and hence led to fewer activated GLP-1 neurons. A corresponding effect was observed in the arcuate pool of CART/POMC neurons with an increased fraction of c-Fos activated neurons in fed untreated animals (veh fed 62 \pm 6%; veh unfed 25 \pm 7; $P<0.05$) which was reduced by liraglutide in the fed condition only (lira fed 36 \pm 4%, $P<0.05$; lira unfed 20 \pm 5).

Conclusion: The data demonstrate that acute administration of liraglutide affects key food regulatory areas in the brainstem but does not lower food intake via the endogenous brain expressed GLP-1 hormone. This indicates that liraglutide gains access to a wide spectrum of GLP-1 receptors in the brain.

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The effect of liraglutide on gastric emptying and body weight is not mediated by vagal afferents nor the area postrema

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Background and aims: Glucagon-Like Peptide-1 (GLP-1) analogues are emerging as important drugs for the treatment of diabetes. Apart from their insulinotropic and glucagonostatic effects GLP-1 analogues also inhibit gastric emptying leading to reduced post-prandial glucose levels, and reduce food intake leading to a decreased bodyweight. It is, however, still unclear which GLP-1 receptors that mediate the effects on food intake and body weight. Also, the gastric emptying mechanism lack to be coupled to a specific receptor expression. GLP-1 receptors are located in several central areas known to be directly involved in energy homeostasis. Since both vagal afferents and area postrema neurons express GLP-1 receptors and are accessible to peripherally circulating GLP-1 we speculated that these receptor populations could mediate the gastric inhibitory and/or the body-weight lowering effects of the once-daily GLP-1 analogue liraglutide. We used vagal deafferentation

and area postrema lesion to identify what receptor populations are the target for liraglutide.

Materials and methods: The study involved 2 groups of 32 male SPD rats further subdivided into 4 groups (veh sham, lira sham, veh surgery, lira surgery). The surgery groups underwent either a selective vagal deafferentiation (SDA, severed left afferent rootlets coupled with a subdiaphragmatic ablation of the posterior trunk) or an area postrema lesion (APx). After 10 days of recovery the acute effect of liraglutide (0.1 mg/kg, sc) on gastric emptying was assessed using an acetaminophen release assay. Hereafter, all animals continued into a 14 or 21 days bi-daily dosing study (0.2 mg/kg, sc).

Results: Interestingly, neither vagal deafferentiation or area postrema lesion affected the ability of liraglutide to inhibit gastric emptying measured as the area under the acetaminophen curves. The AUC's under the acetaminophen exposure curve for the deafferentiation part were 10117 ± 273 (veh sham) vs 2691 ± 798 $\mu\text{g/ml} \cdot \text{min}$ (lira sham) and 9579 ± 760 (SDA veh) vs 3587 ± 990 $\mu\text{g/ml} \cdot \text{min}$ (SDA lira). The AUC's for the area postrema lesions were 6422 ± 365 (veh sham) vs 870 ± 284 $\mu\text{g/ml} \cdot \text{min}$ (lira sham) and 8263 ± 295 (APx veh) vs 3269 ± 466 $\mu\text{g/ml} \cdot \text{min}$ (APx lira). Similarly, the chronic dosing study revealed that liraglutide induced weight loss was independent of vagal deafferentiation. Body weight change was: veh sham 112 ± 1.6 vs lira sham 102 ± 1.1 and SDA veh 112 ± 0.9 vs SDA lira 100 ± 1.1 %. That was also the case for the area postrema lesion: veh sham 119 ± 1.6 vs sham lira 111 ± 0.7 % and APx veh 113 ± 1.4 vs APx lira 108 ± 1.1 %. The completeness of the vagal deafferentiation and AP lesions were verified anatomically and for the vagal deafferentiation functionally as well, by showing that CCK induced anorexia was prevented in vagally deafferented rats.

Conclusion: In conclusion, the data shows that neither vagal nor area postrema GLP-1 receptors mediate the gastric or food intake inhibitory effects of liraglutide, and suggest that other, presumably centrally located, GLP-1 receptors are key mediators of liraglutide induced weight loss.

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Peripheral administration of GLP-1 peptides alters cognition and hippocampal synaptic plasticity in obesity-diabetes

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Background and aims: Studies have shown that high fat feeding in rodents adversely affects cognitive function and hippocampal synaptic plasticity. Interestingly, GLP-1 peptides promote hippocampal progenitor cell proliferation, modulate synaptic plasticity, cross the blood brain barrier and receptors for GLP-1 are expressed in neurons within the hippocampus, neocortex and cerebellum. This study examined effects of peripheral administration of established GLP-1 agonists and antagonists on cognitive function and hippocampal synaptic plasticity in high fat fed mice.

Materials and methods: Swiss TO mice (10–14 per group) maintained on a high fat diet (45% fat, 20% protein and 35% carbohydrate) for 18–20 weeks received twice-daily subcutaneous injections of GLP-1(9–36), exendin(9–39) (each at 25 nmol/kg), Liraglutide (200 $\mu\text{g/kg}$), or saline vehicle (0.9% (w/v) NaCl) over a 28-day period. An additional group of mice ($n=10$) on standard rodent maintenance diet (10% fat, 30% protein, 60% carbohydrate) received twice-daily saline injections. Food intake, bodyweight, non-fasting plasma glucose and insulin concentrations were monitored at 2–3 day intervals. Glucose tolerance (18 mmol/kg; *ip*), general locomotor behaviour, an Object Recognition Task to assess memory and learning and *in vivo* electrophysiological long-term potentiation of hippocampal synaptic transmission were performed at the end of the study.

Results: Mice on high fat diet exhibited increased bodyweight (25%; $P<0.001$), hyperglycaemia (3.6-fold increase; $P<0.001$), hyperinsulinaemia (2.6-fold increase; $P<0.001$) and impaired glucose tolerance (46% increase; $P<0.05$) compared with mice on normal maintenance diet. GLP-1(9–36) and exendin(9–39) did not significantly alter bodyweight, food intake, non-fasting plasma glucose and insulin concentrations or glucose tolerance ($P>0.05$). In contrast, Liraglutide treatment resulted in significant time-dependent reduction in bodyweight (7% reduction), whilst improving plasma insulin (1.5-fold increase; $P<0.05$) and normalizing glucose tolerance (28% glucose reduction; $P<0.05$). GLP-1(9–36), exendin(9–39) and Liraglutide (200 $\mu\text{g/kg}$) did not significantly alter general locomotor activity or anxiety levels. In the Object Recognition Task, mice fed high fat diet exhibited a significant decrease (1.3-fold; $P<0.05$) in recognition index compared to lean controls. While mice treated with GLP-1(9–36) and exendin(9–39) did not affect recognition index, Liraglutide-treated animals displayed a marked increase (1.4-fold; $P<0.05$) in recognition index, indicating improved learning and memory ability. In

analysis of *in vivo* long-term potentiation induced in CA1 region of the hippocampus, long-term potentiation was completely abolished in mice fed high fat diet and there was a tendency for exendin(9–39) therapy to worsen the deleterious effects of high fat feeding on long-term potentiation. Interestingly, daily treatment with Liraglutide ameliorated ($P<0.0001$) the detrimental effects of high fat diet on long-term potentiation.

Conclusion: These data demonstrate that GLP-1 agonists, such as Liraglutide, exert beneficial actions on cognition and synaptic plasticity in mice with high fat diet-induced obesity-diabetes.

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Early undernutrition leads to hypothalamic insulin resistance in adult rats

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Background and aims: It is well known that pathologies such as type II diabetes and cardiovascular diseases are rapidly growing in both western and emergent countries, reaching epidemic proportions. They are strongly linked to obesity. Besides genetic background, several circumstances predispose to obesity, mainly caloric dense foods. Paradoxically, epidemiological studies have shown that exposing to undernutrition during critical periods of growing is also associated with a subsequent increased predisposition to develop obesity and it is known that this precondition of food deprivation is detrimental to some peripheral tissues and its metabolic roles. Previous studies in undernourished animal models have evidenced a rise in the insulin responsiveness in peripheral tissues. Since hypothalamus plays a key role in appetite regulation, it can be considered a prime candidate to be affected by early undernutrition. The aim of this work is to analyze the proteins of hypothalamic insulin pathway and other related proteins in adult rats chronically undernourished from foetal stage.

Materials and methods: Food restriction to Wistar Rats was established from the 14th day of pregnancy following a model previously reported. The animals were anesthetized and they were injected into the third ventricle with 10 mU of insulin in 5 μl saline; only 5 μl saline were administered to basal animals. The rats were killed 15 minutes later, and the hypothalamus was removed and frozen. They were submitted to lysis and next analyzed by the Western blotting method.

Results: The content of the main hypothalamic proteins involved in insulin signalling remained unaltered following undernutrition: IR, IRS1/2, Akt and GSK3. Insulin receptor was phosphorylated largely in undernourished rats than in controls, which can be related with a low PTP1B level in the restricted animals. However, after *icv* insulin, the degrees of GSK3, p70 and ERK phosphorylations did not increase in these rats, in contrast with the controls.

Conclusion: Chronic undernutrition from the foetal stage decreases hypothalamic insulin signalling, which implies a condition of insulin resistance at this level of the central nervous system. The altered hypothalamic insulin pathway could decrease the hormone ability to inhibit feeding in the undernourished population. It is likely that this central adaptation may lead to a higher risk to culminate in obesity whether deprived nutritional environment finished and food became plentiful.

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Unimpaired insulin signalling in hippocampus from insulinopenic Zucker Diabetic Fatty rats

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Background and aims: Type 2 Diabetes Mellitus (T2DM) increases the risk for central nervous system disorders like ischemic stroke, dementia and cognitive deficit. The hippocampus, an important player on learning and memory processes, was recently recognized as presenting a high degree of susceptibility to diabetic complications. Impaired insulin signalling has been implied in the brain pathophysiology under a diabetic setting. The aim of this work is to provide, for the first time, a comprehensive neurotoxic profile as well as

to characterize insulin signalling in hippocampus from Zucker Diabetic Fatty rats (ZDF) rats, a model of T2DM.

Materials and methods: Two groups of male ZDF rats, with 26-weeks-old, were evaluated: Control - ZDF/Gmi +/- (445.70 ± 8.16 g) and Diabetic - ZDF/Gmi fa/fa (363.70 ± 11.80). Metabolic markers including glycemia, insulinemia, glycated haemoglobin and insulin resistance (HOMA-IR) were measured to validate this diabetic model. Functional hippocampal changes were evaluated by analysing the following proteins (western-blot): 1) GFAP - astrogliosis marker; 2) receptor for advanced glycation end products - RAGE; 3) BAX, Bcl-2 - apoptotic markers; 4) syntaxin and SNAP-25 - exocytotic machinery markers and 5) insulin signalization pathway: IRβ; IRS-1; IRS-1 pY⁶¹². $P < 0.05$ was considered as significant (ANOVA and Bonferroni post hoc test).

Results: The diabetic rats showed hyperglycaemia (435.20 ± 15.70 mg/dl; $P < 0.001$), higher levels of glycated haemoglobin (10.96 ± 0.20 %; $P < 0.001$) and insulin resistance index (HOMA-IR: 19.45 ± 0.55; $P < 0.001$) and insulinopenia (0.76 ± 0.15 µg/l; $P < 0.05$) when compared with controls (glycaemia: 140.30 ± 0.92 mg/dl; HbA1c: 3.20 ± 0.14 %; HOMA-IR: 5.73 ± 0.95; insulin: 1.58 ± 0.3 µg/l). Regarding hippocampal markers, no changes in protein expression were found in the diabetic rats when compared with the controls.

Conclusion: This insulinopenic diabetic model shows normal hippocampal insulin signalization pathway as well as lack of hippocampal neurotoxic profile at the studied time-point.

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Relevance of two distinct mechanisms for the antihyperglycaemic action of efaroxan in mice: antagonism to adrenergic α_2 -receptors versus closing of K_{ATP} -channels

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Background and aims: The imidazoline efaroxan seems to exert insulinotropic action via two separate mechanisms. Evidence from isolated islets suggests that only the (+)-enantiomer of efaroxan stimulates insulin release via antagonism at adrenergic α_2 -receptors, while an enantiomer-unspecific mechanism, presumably via closing of K_{ATP} -channels, comes in at higher drug concentrations. The present investigations were to find out, how far each of the two mechanisms holds responsible for antihyperglycaemic effects *in vivo*.

Materials and methods: More than 100 oral glucose tolerance tests (OGTT; 3g/kg) were performed in C57BL mice 45min after oral dosing with racemic efaroxan or with one of its enantiomers (purity > 99.5%). Efaroxan preparations were also compared regarding their effects on hyperglycaemia induced by insulin secretion inhibitors that specifically oppose the mechanisms mentioned above (250mg/kg of the K_{ATP} -channel opener diazoxide, p.o.; or 100µg/kg of the α_2 -receptor agonist UK14,304, i.p.), and regarding their effects on oxygen saturation of hemoglobin in capillary blood from the tail tip (as a surrogate parameter of peripheral vasodilation).

Results: A dose of at least 0.03mg/kg (+)-efaroxan was necessary to reduce the total area under the glucose curve in the OGTT (AUC, % decrease vs. vehicle: 0.01mg/kg, -7±4%, ns; 0.03mg/kg, -11±2%, $p < 0.001$; 0.1mg/kg, -26±2%, $p < 0.001$; 0.3mg/kg, -27±2%, $p < 0.001$; 1mg/kg, -30±2%, $p < 0.001$). Similar results were obtained with racemic efaroxan. A subthreshold dose of the (+)-enantiomer with no effect in the OGTT (0.01mg/kg) was sufficient to counteract hyperglycaemia induced by the α_2 -agonist UK14,304 (AUC, g/l*min: 454±6 vs. 387±19, $p < 0.01$), whereas hyperglycaemia induced by the K_{ATP} -channel opener diazoxide was not affected (516±28 vs. 495±17; ns). This clearly attributes low dose-effects of racemic efaroxan to α_2 -antagonistic properties carried by the (+)-enantiomer. In the OGTTs, (-)-efaroxan exhibited 100-fold lower potency than (+)-efaroxan (AUC, % decrease vs. vehicle: 1mg/kg, -1±4%, ns; 3mg/kg, -9±3%, $p < 0.02$; 10mg/kg, -26±3%, $p < 0.001$). In opposition to (+)-efaroxan, a subthreshold dose of (-)-efaroxan (1mg/kg; no effect in OGTT) counteracted hyperglycaemia induced by diazoxide (AUC, g/l*min: 634±15 vs. 484±21, $p < 0.001$), but not by UK14,304 (434±27 vs. 413±21, ns). This indicates that (-)-efaroxan - at variance to low doses of (+)-efaroxan - acts via a K_{ATP} -dependent mechanism. Oxygen saturation in capillary blood was increased by both the racemate and the α_2 -antagonistic (+)-enantiomer, but not by (-)-efaroxan (% saturation after a dose of 3mg/kg; vehicle, 67±3; racemate, 94±2, $p < 0.001$; (+)-efaroxan, 92±1, $p < 0.001$; (-)-efaroxan, 62±3, ns).

Conclusion: Our results show that efaroxan can reduce blood glucose concentrations in mice via two distinct mechanisms, but glucose lowering via

α_2 -antagonism, as carried by the (+)-enantiomer, occurs at much lower doses than glucose lowering via K_{ATP} -channels. Oxygen saturation of tail blood, which probably reflects vasodilatory activity, was affected only by (+)-efaroxan, hinting at a possible α_2 -related mechanism.

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Carvedilol restores insulin sensitivity in high-sucrose and high-fat diets rats through blockade of the sympathetic nervous system

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Background and aims: Previous results from our lab have shown that chronic caffeine prevents insulin resistance induced by high fat (HF) and high sucrose (HSu) diets in rats through a decrease in sympathetic nervous system activity. To test the hypothesis that blockade of adrenergic activation prevents per se the development of diet-induced insulin resistance we administered carvedilol (CVD), an antagonist of β_1 , β_2 and also α_1 adrenoceptors, to HF and HSu rats.

Materials and methods: Six groups of Wistar rats aged 9-12 weeks were used. The control group was administered the vehicle 0.5% methylcellulose in drinking water (MC). The HSu group was administered a 35% sucrose in drinking water during 28 days. The HF group was fed a lipid rich diet during 21 days. In each group the animals were randomly divided into two subgroups: treated with 0.2g/l carvedilol in 0.5% methylcellulose and not treated. The insulin tolerance test (ITT) was used to measure insulin sensitivity. The effect of CVD treatment on mean arterial pressure (MAP), fasting insulinemia, fasting glycemia, weight gain, visceral fat, serum free fatty acids, cortisol, nitric oxide and catecholamines were also determined.

Results: HSu and HF diets induced insulin resistance and hypertension which were prevented by carvedilol treatment. Carvedilol significantly increased K_{ITT} from 3.35 ± 0.19 to 5.20 ± 0.12% glucose/min in the HSu animals and from 3.49 ± 0.23 to 4.64 ± 0.27% glucose/min in the HF rats ($p < 0.01$). Carvedilol significantly decreased fasting glycemia in the HSuMC group from 116.5±4.56 mg/dl to 91.9±2.75 mg/dl ($p < 0.01$) but not in the HFMC animals. Both HFMC and HSuMC diets caused a significant increase in plasma insulin levels in comparison with the MC group and this effect was reversed by CVD administration (MC=2.45±0.26µg/l; HFMC=4.88±0.30µg/l; HSuMC=4.36±0.37µg/l; HFCVD= 2.42±0.42 µg/l; HSuCVD=1.76±0.79µg/l; $p < 0.001$). Carvedilol decreased MAP from 113.5±3.2 to 99.0±5.2 mmHg and from 113.0±4.1 mmHg to 99.4±4.6 mmHg in the HSu and HF models, respectively ($p < 0.01$). The effect of the drug on insulin sensitivity was correlated neither to changes in weight gain, visceral fat mass, serum free fatty acids, cortisol, nitric oxide nor circulating catecholamines.

Conclusion: We concluded that the activation of the sympathetic nervous system is involved in the pathogenesis of diet-induced insulin resistance and high blood pressure in HSu and HF rats and that adrenergic blockade prevents these deleterious effects.

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Gastrointestinal-mediated glucose disposal in vagotomised subjects

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Background and aims: After secretion, the incretin hormone glucagon-like peptide-1 (GLP-1) is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4), resulting in less than 15% of the intact biologically active peptide reaching the systemic circulation. This has led to the hypothesis that GLP-1 acts locally, by activating afferent sensory nerve fibers of the vagus nerve before being degraded. We aimed to evaluate the role of vagal

transmission for the effect of GLP-1 on gastrointestinal-mediated glucose disposal (GIGD).

Materials and methods: Eight truncally vagotomised with pyloroplasty (due to duodenal ulcer) subjects (age: 70 ± 2 years (mean \pm SEM); BMI: 24 ± 1 kg/m²; fasting plasma glucose (FPG): 5.8 ± 0.2 mmol/l), 7 subjects previously treated for oesophageal cancer with resection of the cardia including truncal vagotomy and pyloroplasty (age: 64 ± 2 years; BMI: 23 ± 1 kg/m²; FPG: 5.4 ± 0.1 mmol/l) and 5 healthy control subjects (age: 68 ± 2 years; BMI: 25 ± 1 kg/m²; FPG: 5.4 ± 0.2 mmol/l) were examined on three separate occasions: 4h 50-g OGTT, isoglycaemic iv glucose infusion (IIGI) and an additional OGTT with concomitant DPP-4 inhibition.

Results: Isoglycaemia during IIGIs was obtained using 24 ± 2 g and 28 ± 4 g of glucose in subjects with truncal vagotomy associated with surgery for duodenal ulcer and cardia resection, respectively ($p = \text{NS}$), and 17 ± 2 g in healthy control subjects ($p < 0.05$), resulting in GIGD [$100\% \times (\text{glucose}_{\text{OGTT}} - \text{glucose}_{\text{IIGI}}) / \text{glucose}_{\text{OGTT}}$] of 51 ± 4 and $45 \pm 8\%$ in the two vagotomised groups (NS) and $67 \pm 4\%$ in the healthy control subjects ($p < 0.05$). Peak concentrations of plasma glucose were similar in the two vagotomised groups (13.6 ± 0.9 vs 13.3 ± 0.8 mmol/l, $p = \text{NS}$), and higher than in the control group (9.1 ± 0.5 mmol/l, $p < 0.003$). There was no effect of DPP-4 inhibition on PG in any group.

Conclusion: Vagotomised subjects have impaired glucose homeostasis, which may be due to accelerated gastric emptying. Interestingly, GIGD was diminished in these subjects, suggesting that intact vagal innervation is important for the maintenance of normal glucose homeostasis.

Clinical Trial Registration Number: H-A-2009-060

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Conclusion: PRED treatment dose-dependently impaired pancreatic islet-cell function in healthy men, in addition to reduced whole body insulin sensitivity. GC-induced alterations in sympathovagal balance may contribute to their adverse effects on islet-cell function.

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Glucocorticoid-induced islet cell dysfunction: a potential role for altered sympathovagal balance?

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Background and aims: Glucocorticoids (GCs) are frequently used anti-inflammatory agents. Prolonged use, however, may induce insulin resistance, glucose intolerance and might eventually result in diabetes. The effects of GCs on pancreatic islet-cell function and the causal mechanisms remain more controversial. Here, we hypothesized that the GC prednisolone (PRED) impairs islet-cell function and that alterations of autonomous nervous system (ANS) balance may contribute to these changes.

Materials and methods: We conducted a double-blind randomised placebo-controlled dose-response trial in which 32 healthy normoglycaemic males (age: 21.0 ± 2.1 years, BMI: 21.9 ± 1.7 kg m⁻²) were treated with either PRED 30 mg q.d. (PRED30), PRED 7.5 mg q.d. (PRED7.5) or placebo (PLB) for 14 days. At baseline and day 14 of treatment, a combined hyperinsulinaemic-euglycaemic and hyperglycaemic clamp with additional arginine stimulation was performed, to assess whole body insulin sensitivity and beta-cell function. Moreover, fasting glucagon levels were determined as a measure of alpha-cell function. Finally, ANS function was assessed by measurement of heart rate variability and subsequent power spectral analysis. HF_{norm} was calculated as a measure of parasympathetic activity, the ratio between LF_{norm} and HF_{norm} (LF/HF ratio) was taken to represent the sympathovagal balance.

Results: PRED dose-dependently decreased insulin sensitivity as compared to PLB: mean differences: -2.1 ± 0.8 mg kg⁻¹ min⁻¹ ($P = 0.021$) for PRED7.5 and -4.5 ± 0.7 mg kg⁻¹ min⁻¹ ($P < 0.001$) for PRED30. PRED tended to increase the beta-cell function parameters 1st- and 2nd-phase glucose-stimulated C-peptide secretion ($\beta = 305$; $P = 0.098$) and ($\beta = 311$; $P = 0.089$) respectively; this effect was abolished when adjusted for insulin sensitivity. C-peptide secretion following arginine stimulation on top of hyperglycaemia ($\text{ASI-iAUC}_{\text{CP}}$) was reduced both by PRED7.5: -2.7 (-5.2 - 0.3) nmol/l•min, $P = 0.05$ and by PRED30: -3.0 (-7.6 - 0.2) nmol/l•min, $P = 0.02$. Fasting glucagon levels were dose-dependently increased: 2.2 ± 0.9 pmol/l ($P = 0.04$) for PRED7.5 and 4.3 ± 1.2 pmol/l ($P = 0.003$) for PRED30. Concerning ANS function, PRED treatment tended to decrease HF_{norm} ($\beta = -325$; $P = 0.08$). Overall, changes in HF_{norm} associated with fasting plasma glucose levels ($\beta = -0.407$; $P = 0.03$) and inversely correlated with fasting glucagon levels ($\beta = -0.337$; $P = 0.07$). The change in LF/HF ratio was negatively associated with the beta-cell function parameter $\text{ASI-iAUC}_{\text{CP}}$ ($\beta = -365$; $P = 0.05$).

PS 039 Fatty liver

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Plasma α -hydroxybutyrate and linoleoyl-glycerolphosphocholine as new markers of insulin resistance and fatty liver disease

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Background and aims: Recently, we have shown that increased levels of plasma α -hydroxybutyrate (α -HB) and decreased levels of plasma linoleoylglycerophosphocholine (L-GPC) are associated with peripheral insulin resistance (measured by the euglycemic hyperinsulinemic clamp technique). Since both α -HB and L-GPC are produced in the liver, with α -HB levels directly related to the rate of hepatic glutathione synthesis, the goal of this study was to test if α -HB and L-GPC are markers for fatty liver disease.

Materials and methods: In the RISC population (1,222 nondiabetic, normotensive subjects, age 44 ± 8 years (mean \pm SD) [range 30–60], BMI 25.5 ± 4.1 kg/m², [range 16.9–43.9]), we measured circulating α -HB concentrations, liver enzymes (ALT, AST, GGT), and plasma L-GPC levels. Fatty liver accumulation was assessed using the recently validated fatty liver index, FLI. Hepatic insulin resistance (Hep-IR) was estimated in 380 subjects as the product of endogenous glucose production (by ²H₂-glucose infusion) and fasting plasma insulin. To assess L-GPC levels directly in the liver, open liver biopsies were performed in the fasting state during elective cholecystectomy from lean subjects without DM2 (n=8) and during gastric bypass from obese subjects with or without DM2 (n=25).

Results: After adjusting for center, sex, and age by multiple linear regression analysis, α -HB was directly related to FLI (partial $r = 0.21$, $p < 0.0001$), whereas L-GPC was reciprocally related to FLI (partial $r = -0.32$, $p < 0.0001$). Furthermore, a high α -HB and a low L-GPC both contributed to a high FLI value independently of center, sex, and age. In the subgroup with tracer measurements - and after adjustment for center, sex, age, and BMI - L-GPC was also reciprocally related to Hep-IR (partial $r = -0.12$, $p = 0.02$). In support of these findings, we further found that hepatic L-GPC were 5-fold lower in the obese group compared to lean controls.

Conclusion: Raised plasma concentrations of α -HB and L-GPC mark for the presence of fatty liver independently of sex and age. Low L-GPC levels are also specifically associated with Hep-IR. Liver L-GPC levels were also found to be significantly lower in obese and T2D subjects.

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Nonalcoholic fatty liver disease markers and insulin resistance in type 1 diabetes

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) has been associated with the insulin resistance. The aim of this study was to explore the relationship between markers of NAFLD, including concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK), γ -glutamyltransferase (GGT), ferritin and bilirubin, and insulin resistance in type 1 diabetes.

Materials and methods: Three hundred and 53 patients with type 1 diabetes were included. None showed signs of adrenal, thyroid, renal, liver or cardiovascular diseases and received drugs, apart from insulin, that could attenuate glucose metabolism, insulin sensitivity or liver function. Insulin sensitivity was measured by estimated glucose disposal rate (eGDR) calculated with

the equation: $eGDR = 24.31 - (12.22 \times WHR) - (3.29 \times HT) - (0.57 \times HbA1c)$. The units were $mg \cdot kg^{-1} \cdot min^{-1}$; WHR=waist to hip ratio; HT=hypertension. Lower eGDR levels indicated greater insulin resistance. Correlations and multiple logistic regressions analysis were performed to identify relationships between NAFLD markers and eGDR, individual components of insulin resistance and risk of insulin resistance.

Results: AST, ALT, AST-to-ALT ratio, ALK and ferritin significantly correlated with insulin resistance measured by eGDR ($r = -0.13, -0.14, 0.13, -0.18$, and -0.24 respectively, all $p < 0.05$). Subjects in the upper quartile of the eGDR had significantly reduced levels of AST (median 19 vs 22 U/L, $p = 0.007$), ALT (median 18 vs 23 U/L, $p < 0.001$), ALK (median 61 vs 73 U/L, $p < 0.001$), GGT (median 14 vs 19 U/L, $p < 0.001$) and ferritin (median 31 vs 100 $\mu g/L$, $p < 0.001$), and significantly increased levels of AST-to-ALT ratio (median 1.0 vs 0.88, $p = 0.02$) compared to patients in the lowest quartile. In a multiple logistic regression models adjusted for age, sex, duration of diabetes and BMI, higher AST, ALT and ALK levels was associated with an increased risk for development of insulin resistance (OR=1.03, 1.02, and 1.01 respectively, all $p < 0.05$). The risk of insulin resistance increased by a factor of 1.77 for subjects in the 4th quartile of AST, 2.61 for ALT and 2.68 for ALK, compared to those in the 1st quartile.

Conclusion: We demonstrated that NAFLD markers are associated with insulin resistance measured by clinical parameters (eGDR) in type 1 diabetes. After adjustment for covariates, ALT, AST and ALK were independent predictors of insulin resistance. These findings indicate that an increased level of ALT, AST and ALK could represent an additional marker of insulin resistance in type 1 diabetic patients who might be considered at high risk for the development of hepatic and other manifestations of insulin resistance syndrome.

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Hepatocyte SHP-1, a key PTPase in the development of diet-induced insulin resistance

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Background and aims: We have previously reported that the SH2 domain-containing protein tyrosine phosphatase 1 (SHP-1) is expressed in both liver and skeletal muscle and modulates glucose homeostasis by negatively interfering with insulin signaling. However, the potential role of SHP-1 in obesity-linked insulin resistance and type 2 diabetes (T2D) has not yet been examined. Here we have confirmed that **Materials and methods:** SHP-1 is upregulated in the livers of obese insulin-resistant mice fed a high-fat diet (HFD) for 8 weeks as compared to mice on standard chow (SD). Given that SHP-1 is expressed in several cell types in liver tissue, notably hepatocytes, Kupffer cells, macrophages, and endothelial cells, the precise role of hepatocyte SHP-1 in promoting insulin resistance and T2D was investigated by generating hepatocyte-specific SHP-1 knockout mice (Heshp1KO) using the Cre/LoxP system. Both Heshp1KO and their SHP-1 LoxP-containing littermates (WT) were fed SD or HFD for 8 and 16 weeks before metabolic phenotyping. Even on a SD diet, Heshp1KO mice already exhibited improved glucose tolerance and lower fasting glycemia.

Results: After HFD-feeding for 8wk and 16wk, although the glucose tolerance was comparable in both groups of mice, the Heshp1KO mice were significantly less hyperinsulinemic with consistently lower fasting glycemia when compared to their WT counterparts. Fast-refeeding experiments also showed reduced diet effect on post-prandial levels of glucose, triglyceride, and cholesterol in Heshp1KO mice. These metabolic improvements occurred without any alterations in body weight gain, fat composition, or food intake between Heshp1KO mice and their WT littermates. To specifically assess hepatic versus peripheral insulin sensitivity, we also conducted hyperinsulinemic-euglycemic clamp studies and found that the ability of insulin to suppress liver glucose output was improved in Heshp1KO mice as compared to their WT controls on either diet. However, no significant changes were observed for insulin-dependent peripheral glucose disposal. Consistent with the metabolic data, insulin-mediated Akt S473 and T308 phosphorylation was impaired in both liver and isolated hepatocytes of HFD-fed WT mice, but this defect was prevented in HFD-fed Heshp1KO animals.

Conclusion: These results provide strong evidence that hepatocyte SHP-1 is a major negative regulator of insulin signaling and glucose metabolism, contributing to the development of hepatic insulin resistance in obesity. This research is supported by CIHR.

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Activity of hepatic AMP-activated protein kinase in type 2 diabetes mellitus and human nonalcoholic fatty liver disease

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Background and aims: The AMP-activated protein kinase (AMPK) represents an ubiquitously expressed molecular fuel sensor that coordinates anabolic and catabolic pathways in response to cellular stress. However, no previous studies have investigated hepatic AMPK protein in humans. The aim of the current study was to evaluate the regulation of AMPK at the mRNA and protein level in the context of nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM). To assess AMPK activity, phosphorylation at Thr172 at the catalytic alpha subunit and activation of AcetylCoA-Carboxylase (ACC) as a direct downstream phosphorylation target were investigated.

Materials and methods: 30 well characterized participants undergoing partial hepatectomy were enrolled into a cross-sectional study, n=11 metabolically healthy subjects and n=19 suffering from a pathologic glucose metabolism (Impaired Fasting Glucose, n=6; Metabolic Syndrome, n=7; T2D, n=6). Liver tissue was sampled intrasurgically under perfused conditions to avoid metabolic artifacts due to hypoxia. Hepatic steatosis and inflammation was determined by histological evaluation and liver fat content was quantified by measuring liver tissue triacylglycerol (TAG). Transcription levels of selected AMPK isoforms were determined by quantitative real time PCR and alphaAMPK protein, Thr172 phosphorylation, ACC1, and ACC activity were analyzed by means of Western immunoblotting.

Results: Patients with pronounced hepatic steatosis revealed significantly higher measured TAG levels ($P=0.004$) and a higher NASH Activity Score (NAS, $P<0.001$) as compared to patients with $<10\%$ liver fat. Estimated insulin sensitivity was negatively associated with the NAS ($r^2=-0.44$; $P=0.027$). No significant effect of pronounced liver steatosis on hepatic transcript levels of selected AMPK subunits was observed ($P>0.05$, respectively). However, liver alphaAMPK protein ($P=0.013$), and phosphorylation at Thr172 ($P=0.027$) was significantly reduced with hepatic steatosis. A significant reduction of alphaAMPK protein levels ($P=0.013$) and Thr172 phosphorylation ($P=0.011$) was further detected in patients suffering from T2D vs. subjects with normal glucose metabolism. No significant differences were found concerning either hepatic ACC1, nor Ser79 phosphorylated ACC ($P>0.05$, respectively).

Conclusion: AMPK activity and protein levels were reduced with hyperglycemia and hepatic steatosis in human liver. According to the results of the current study, AMPK apparently underlies an extensive posttranslational regulation in human liver tissue, while ACC showed no significant differences.

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In vivo postprandial lipid handling in liver and muscle of diabetic rats is disturbed

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Background and aims: Intracellular lipid (ICL) accumulation in liver and muscle is strongly related to the development of insulin resistance and type 2 diabetes (T2D). It is unknown, however, whether excessive lipid accumulation is a consequence of an increased lipid uptake, a decreased lipid utilization, or a more structural imbalance between uptake and utilization. We applied a ^1H - ^{13}C magnetic resonance spectroscopy (MRS) method in combination with the administration of ^{13}C -labeled lipids in a rat model of T2D to study *in vivo* lipid handling in insulin-resistant liver and muscle at different stages in the pathogenesis of T2D.

Materials and methods: Four groups of n=6 male Zucker diabetic fatty rats were used for this study: obese, pre-diabetic fa/fa rats and lean, non-diabetic fa/+ littermates at the age of 6 weeks, and obese, diabetic fa/fa rats and lean, non-diabetic fa/+ littermates at the age of 12 weeks. ^1H - ^{13}C MRS measurements were performed in liver and tibialis anterior muscle at baseline and 4, 24 and 48 h after oral administration of 1.5 g $[\text{U-}^{13}\text{C}]$ Algal lipid mixture per kg body weight. Total and ^{13}C -labeled ICL content were determined from the MR spectra. Statistical analysis was performed using ANOVA for repeated measures (SPSS).

Results: At baseline, total ICL content was higher in fa/fa rats compared with fa/+ rats in both liver (Figure 1A) and muscle (Figure 1B), and at both ages. Total ICL content did not change after the administration of ^{13}C -labeled lipids. Both in pre-diabetic and in diabetic fa/fa rats, hepatic lipid uptake was increased compared with non-diabetic fa/+ rats, whereas the depletion of ^{13}C -labeled lipids in the liver did not seem to be largely affected (Figures 1C and 1D). Likewise, in muscle of diabetic fa/fa rats, lipid uptake was higher than in muscle of fa/+ rats (Figure 1F). In contrast, lipid uptake in muscle of younger, pre-diabetic fa/fa rats was lower than in controls (Figure 1E). In muscle of both pre-diabetic and diabetic fa/fa rats, ^{13}C -labeled lipid depletion appeared to be impaired compared with controls.

Conclusion: In both insulin-resistant liver and muscle of fa/fa rats, postprandial lipid handling was found to be disturbed when compared with controls. In the pre-diabetic state, muscle appeared to be protected from massive lipid uptake, whereas lipid uptake in the liver was largely increased. In contrast, after developing full-blown diabetes, lipid uptake was highly elevated in both liver and muscle.

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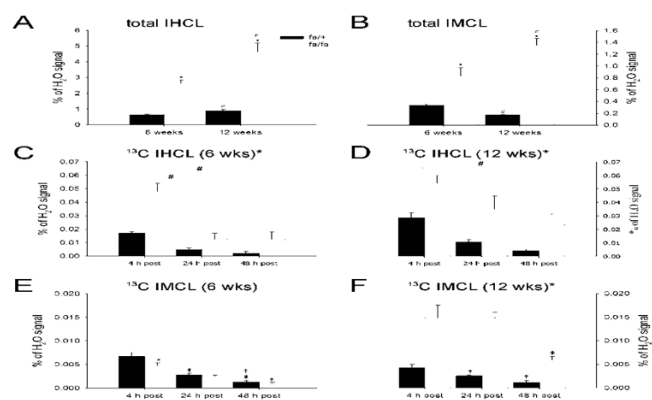


Figure 1. Total intracellular lipid content in liver (IHCL, A) and muscle (IMCL, B) at baseline and ^{13}C enrichment of IHCL (C and D) and IMCL (E and F) at the age of 6 weeks (C and E) and 12 weeks (D and F). Data are expressed as mean percentages of the unsuppressed water signal \pm SEM. * (Fa/fa) significantly different from fa/+ ($p < 0.05$); # significantly different from 6 weeks ($p < 0.05$); † significantly different from 4 h post ($p < 0.05$); ‡ significantly different from 24 h post ($p < 0.05$).

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Resistance exercise improves liver fat and glucose control in people with non-alcoholic fatty liver disease

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Background and aims: The accumulation of fat in the liver is a direct cause of insulin resistance and is central to the pathogenesis of Type 2 diabetes. Non-alcoholic fatty liver disease (NAFLD) is also associated with an increased risk of cardiovascular disease and advanced liver disease. Lifestyle interventions remain the cornerstone of NAFLD management. However, the evidence underpinning the impact of exercise on liver fat and its mediators is poorly characterized. This is the first study to define the effect of resistance exercise on liver fat and glucose control in adults with fatty liver disease.

Materials and methods: 19 adults with NAFLD were randomised to 8 weeks resistance exercise ($n = 11$) or standard care ($n = 8$). Liver fat, glucose control, lipid oxidation during sub-maximal exercise, and abdominal fat were assessed respectively by ^1H -magnetic resonance spectroscopy, frequently sampled oral glucose tolerance test (fsOGTT), indirect calorimetry and MRI before and after the intervention. The exercise group undertook resistance training 3 times per week.

Results: There was no change in body weight in either group (0.1 ± 0.4 vs. $0.9 \pm 0.7\%$ change, $p > 0.05$). Liver fat was significantly reduced with exercise but not in control (17 ± 3 vs. 15 ± 3 vs. 17 ± 3 to $17 \pm 3\%$ HTGC, $p < 0.05$). Resistance exercise significantly improved fasting glucose (6.0 ± 0.6 to 5.2 ± 0.3 vs. 5.9 ± 0.8 to 6.4 ± 1.0 mmol/L, $p < 0.05$) and glucose area under the curve during the fsOGTT compared with control (-10 ± 3 vs. $11 \pm 6\%$ change, $p < 0.01$). Fat oxidation, shown by a reduced RQ and assessed during 60 minutes sub-maximal exercise, was significantly increased in exercise but not control (RQ 0.93 ± 0.0 to 0.90 ± 0.0 vs. 0.90 ± 0.0 to 0.89 ± 0.0 , $p < 0.05$). There was no change in visceral (3 ± 3 vs. $-4 \pm 4\%$ change, $p > 0.05$) or subcutaneous (-2 ± 2 vs. $2 \pm 2\%$ change, $p > 0.05$) fat in either group.

Conclusion: Resistance exercise offers a time efficient alternative to aerobic exercise and is effective in decreasing liver fat content, increasing fat oxidation and improving glucose control in adults with NAFLD, independent of body weight or abdominal obesity.

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Exercise as a treatment strategy for non-alcoholic fatty liver disease: impact on vascular health and hepatic steatosis

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is characterised by the accumulation of triglycerides in the liver and is associated with liver-related morbidity and mortality. Nevertheless, the leading cause of death in these patients is cardiovascular disease (CVD). Flow mediated dilation (FMD) provides information regarding endothelial cell health and is an early marker of CVD which is directly linked to the risk of myocardial infarction. The efficacy of exercise training in reducing hepatic fat in NAFLD patients is poorly characterised and its impact on vascular function has not previously been reported. We hypothesised FMD would be impaired in NAFLD and that exercise would reduce hepatic fat and improve FMD.

Materials and methods: Eleven NAFLD patients and ten healthy controls volunteered. Whole body magnetic resonance imaging quantified visceral and subcutaneous fat and ¹H magnetic resonance spectroscopy determined hepatic fat. Brachial artery FMD, cardiorespiratory fitness and fasting glucose, lipids and alanine transaminase (ALT) were assessed in all subjects. All NAFLD patients then underwent a 16-week supervised exercise intervention (moderate intensity, 30–45 min, 3–5 times per week) after which all assessments were repeated. Differences between NAFLD and controls, and the changes with exercise were analysed using t-tests. Data are presented as mean±SE.

Results: NAFLD and controls were matched for age (49±4 vs 46±3 yrs; P=0.50) and BMI (30±1 vs 29±1 kg/m²; P=0.50). Hepatic (22.3±5.1 vs 2.6±0.7%; P=0.003) and abdominal visceral (6.0±0.8 vs 3.3±0.4 l; P=0.006) fat was elevated and FMD was impaired (5.2±0.8 vs 9.1±1.0%; P=0.01) in NAFLD patients when compared to controls. Following the exercise intervention, hepatic fat decreased by 30% (22.3±5.1 vs 15.7±3.6%; P<0.004) and FMD significantly improved (5.2±0.8 vs 8.0±0.6%, P=0.002) in NAFLD patients. This was accompanied by a reduction in abdominal subcutaneous fat (8.0±0.8 vs 7.6±0.8 l; P=0.02) and significant improvements in fitness ALT, BMI, body mass, hip and waist circumference (P<0.003). No changes were evident in lipid profiles, visceral or total subcutaneous fat following exercise.

Conclusion: This is the first study to demonstrate the therapeutic effects of exercise training on hepatic steatosis and vascular health in NAFLD patients. Currently, there is no effective pharmacological treatment to decrease hepatic fat in this group at high risk of CVD. Therefore, these data strongly support the efficacy of exercise training as a leading preventive strategy in NAFLD.

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PS 040 Exercise and diabetes

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Habitual physical activity is associated with physical fitness but not with glycaemic control in people with type 1 diabetes

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Background and aims: Regular daily physical activity is encouraged by clinicians as part of the patient's diabetes management. It is however unclear if physical activity protects the individual from diabetes-related complications. Increased levels of physical fitness reduce the risks of macrovascular disease and improve microvascular function, while better glycaemic control reduces the risk of microvascular dysfunction. In our study, we examine the association between habitual physical activity, physical fitness and glycaemic control in a free-living cohort of people with type 1 diabetes.

Materials and methods: 23 adults (12 female) with type 1 diabetes have been studied (age=37±11y, BMI=26.5±5kg.m⁻², diabetes duration=16±11y, HbA_{1c}=7.7±1.3%, mean±SD) in an open, non-randomised, uncontrolled trial. Habitual physical activity was monitored by a SenseWear Pro2 armband (BodyMedia, USA), while glycaemic control was assessed by the Guardian Real-Time Continuous Glucose Monitoring System (CGMS) (Medtronic, USA) for up to 12 days (8±3 days), to obtain a 'snapshot' of the individual's daily lifestyle. The participants were free to partake in any activity, and decide on any therapeutic decision which affected their blood glucose. Physical fitness was quantified by the BMI, percentage body fat measured by DEXA scan, muscle strength measured by hand dynamometer and maximal oxygen consumption (VO_{2max}) attained while exercising on a treadmill under the Bruce protocol. Resting muscle blood flow (Q_r) was also measured by venous occlusion plethysmography on the leg. Pearson correlation coefficients are quoted.

Results: There was an association between the average daily total energy expenditure (TEE) and BMI (r=-0.63, p=0.001), percentage body fat (r=-0.67, p=0.0004), muscle strength (r=0.41, p=0.05) and VO_{2max} (r=0.66, p=0.0009; adjusted for age). The percentage average daily time spent performing moderate intensity activities, defined here to be between 3 to 6 METs was correlated with BMI (r=-0.49, p=0.02), percentage body fat (r=-0.60, p=0.002) and VO_{2max} (r=0.63, p=0.002; adjusted for age). No association was found between TEE and daily mean blood glucose (r=-0.20, p=0.36) or daily glycaemic variability quantified by: a) the blood glucose standard deviation (r=0.07, p=0.76) and b) the coefficient of variation (standard deviation normalised by the mean) (r=0.23, p=0.28). There was also no association between TEE and resting Q_r (r=0.11, p=0.64).

Conclusion: Using an objective quantitative measure to assess daily energy expenditure in free living subjects with type 1 diabetes, we have shown that increased levels of habitual physical activity are associated with better cardio-respiratory fitness. Surprisingly, no association was shown between habitual physical activity and a measure of continuous glycaemic control. This finding may be attributable to individuals' extra carbohydrate ingestion to avoid physical activity-induced hypoglycaemia causing hyperglycaemia. We speculate that although increased levels of physical activity energy expenditure confer protection against macrovascular disease, our data suggest that increased levels of physical activity energy expenditure may have little impact on risk of microvascular disease because habitual physical activity had little effect on glycaemia in people with type 1 diabetes.

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Influence of exercise on phenotypic characteristics and metabolomic response in patients with type 1 diabetes

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Background and aims: Regular exercise in people with type 1 diabetes (T1D) does not necessarily lead to improved control. Indeed, the metabolic distur-

bances associated with sustained exercise may lead to worsening control unless great care is taken to adjust carbohydrate intake and insulin dosage. Metabolomic is becoming widely spread used as a new and powerful tool for discerning significant changes at metabolic level. In this study we wanted to identify: 1) the effects of regular exercise practice on phenotypic characteristics, and 2) the impact of acute exercise on the serum metabolome of patients with T1D compared to controls.

Material and methods: A total of 45 type 1 diabetic patients without chronic complications and 45 controls were included. From each group individuals were classified as non competitive athletes (≥ 3 sessions of exercise per week) or sedentary. We obtained data of fitness levels (maximal test on a cycle-ergometer), body composition (DEXA), glycemic control and dietary intake. Further, 10 male with T1D (35.1 ± 2.7 years old) and 11 controls (32.7 ± 2.8 years old) with similar cardio-respiratory capacity ($VO_{2\max}$ 33.4 ± 7.1 mL $\text{kg}^{-1} \cdot \text{min}^{-1}$ vs. 33.9 ± 9.1 , respect.) were selected to perform an acute test of exercise (30 min of cycle-ergometer at 80% of $VO_{2\max}$). Fasting serum samples were withdrawn prior and at the end of the exercise and were analysed using two different platforms: ^1H NMR and gas chromatography- mass spectrometry.

Results: In the table below are summarized the phenotypic characteristics of the total population. Athletes (T1D and controls) showed better fitness capacity and lower total and abdominal fat, comparing to sedentary groups. In type 1 diabetes group, athletes elicit a tendency to better HbA1c (7.3 ± 1.2 vs. 7.7 ± 1.3) higher total energy intake (2096 ± 434 vs. 1832 ± 466 kcal/day) and a significant increase in carbohydrate consume (200 ± 52 vs. 161 ± 56 g/day, $p=0.02$) in comparison to sedentary counterparts. In the acute exercise test, T1D presented elevated levels of insulinemia before (18.6 ± 14.6 UI/L) and after 30 min (25.1 ± 21.5). In the untargeted metabolomic analysis we identified significant increments of indicators of tricarboxylic acid cycle (malate, fumarate, succinate, citrate, α -ketoglutarate), lypolysis (glycerol) and fatty acid oxidation (oleic acid, palmitoleic acid, linoleic acid) in both groups, greater in control than in diabetes.

Conclusions: Subjects with T1D and controls presented similar metabolic characteristics, and the blunted response to exercise in T1D group is probably consequence of hiperinsulinemia due to insulin treatment.

	Type 1 diabetes		Controls	
	Athletes n=23	Sedentary n=22	Athletes n=24	Sedentary n=21
Sex	17/6	15/7		
Age (years)	35.0 \pm 10.8	41.5 \pm 12.1	32.5 \pm 8.1	35.2 \pm 8.3
Diabetes Evolutions (years)	16.5 \pm 12.0	19.1 \pm 8.7	--	--
BMI (kg/m ²)	24.0 \pm 2.8	25.5 \pm 3.6	24.3 \pm 2.6	24.6 \pm 2.8
Fitness level				
$VO_{2\max}$ (mL O ₂ /kg/min)	39.6 \pm 7.2	22.8 \pm 5.8*	41.9 \pm 10.1	28.1 \pm 6.5*
IPAQ questionnaire (MET/min/week)	3071.3 \pm 1737.0	1398.3 \pm 961.5*	2698.3 \pm 1547.8	1345.5 \pm 987.6*
Body composition				
Total body fat (%)	22.9 \pm 7.1	32.2 \pm 8.0*	23.3 \pm 5.6	34.2 \pm 8.1*
Abdominal fat (%)	25.2 \pm 10.4	36.6 \pm 9.7*	26.9 \pm 9.9	39.8 \pm 10.1**

Data expressed as mean \pm SD. Level of significance $p < 0.05$. a: $p < 0.05$ vs. Type 1 diabetes athletes; b: $p < 0.05$ vs. controls athletes; c: $p < 0.05$ vs. Type 1 diabetes athletes

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Increased skeletal muscle mitochondrial respiration in patients with type 2 diabetes following dietary and exercise interventions

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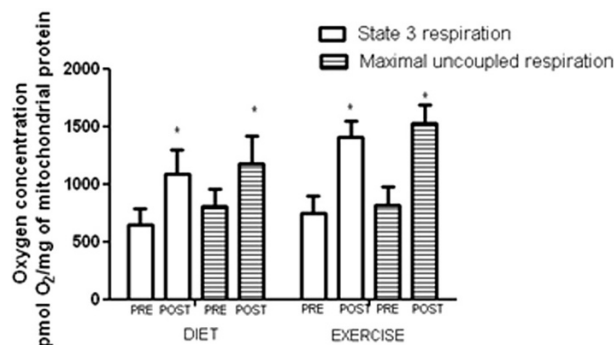
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Background and aims: Type 2 diabetes is characterized by insulin resistance, associated with mitochondrial dysfunction in skeletal muscle. Diet-induced weight loss and aerobic exercise intervention lead to improvements in insulin sensitivity. We compared mitochondrial respiration in muscle biopsies from sedentary patients with type 2 diabetes following either diet or exercise intervention.

Materials and methods: 12 patients with type 2 diabetes (mean age 44.4 ± 3.15 years; BMI 36.4 ± 1.8 kg/m²; weight 108.1 ± 6.6 kg) participated in either intervention, four of whom completed both. Both diet and exercise were designed to cause a 2,500 calorie deficit per week. High resolution respirometry was used to measure oxygen flux capacity in isolated mitochondria from vastus lateralis muscle biopsies taken pre and post interventions. Results represent mean \pm SEM. Non-parametric tests were used for statistical analysis.

Results: Aerobic training significantly increased maximal oxygen consumption (2.6 vs. 3.0 L/min; $p=0.008$) and reduced fat mass (44.9 vs. 42.6 kilograms; $p=0.011$). Aerobic training resulted in increased mitochondrial state 3 respiration (749.1 vs. 1408.5 pmol O₂/mg protein; $p=0.036$) and maximal uncoupled respiration (818.4 vs. 1531.5 pmol O₂/mg protein; $p=0.036$). Following the dietary intervention, subjects lost weight (111.8 vs. 106.8 kg; $p=0.028$) and fat mass (50.0 vs. 46.8 kg; $p=0.046$), while $VO_{2\max}$ was unchanged (2.26 vs. 2.34 L/min; $p=0.39$). Dietary intervention resulted in increased mitochondrial state 3 respiration (642.2 vs. 1085.9 pmol O₂/mg protein; $p=0.018$) and maximal uncoupled respiration (803.1 vs. 1172.7 pmol O₂/mg protein; $p=0.028$). Insulin sensitivity measured by OGIS was 303.0 and 352.7 pre and post exercise, 290 and 318 pre and post diet, not reaching statistical significance in either group.

Conclusion: Skeletal muscle mitochondrial respiration is substantially improved in obese sedentary patients with type 2 diabetes by both diet and aerobic exercise, and to a greater extent with exercise.



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Resistance alone or combined resistance plus aerobic exercise training increases IRS-1 expression in muscle of type 2 diabetic subjects

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Background and aims: Exercise training is known to improve insulin sensitivity and recent research has reported that combined exercise can be the most effective modality. The purpose of this study was to compare the effects of different exercise training on IRS-1, GLUT-4, JNK2, NFkB tissue expression and IKK fosforilation on skeletal muscle of type 2 subjects.

Materials and methods: Forty eight type 2 diabetics were randomly assigned to three groups of training (3 times/week, 60 min/session) designated as aerobic group (n=12), resistance group (n=12), combined group (n=12) and a control group (n=12). Inclusion criteria was being type 2 diabetes according ADA diagnostic criteria, age between 30 and 70 years old and BMI ranging from 25 and 40 kg/m². Exclusion criteria include current insulin therapy, conditions that could preclude physical activity and corticosteroid use. Muscle microbiopsies were performed before and after training (between 60 and 96 hours after the last bout of exerciser) to quantify IRS -1, GLUT-4, JNK2, NFkB expression and IKK fosforilation on skeletal muscle.

Results: After training GLUT-4, JNK2, NFkB expression and IKK fosforilation did not change but IRS-1 expression increased by 65% in the resistance ($p < 0.05$) and by 89,7% in the combined group ($p < 0.01$). We used the analysis of variance (Two-way ANOVA) to assess significant differences between the groups and bonferroni post-tests to compare the mean before and after the training.

Conclusion: The increased IRS-1 expression on skeletal muscle of type 2 diabetes persists longer than sixty hours after training on the resistance and combined group despite a normal GLUT-4 muscle expression. The inhibitory effect of exercise on inflammatory pathways like JNK2, NFkB expression and IKK fosforilation was not seen 60 to 96 hours after the last bout of exercise. This persistent effect of resistance and combined exercise training on IRS-1 expression could be responsible for the best effect related on improvements of insulin signaling transduction in these modalities of exercise.

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High altitude trekking preceded by training improves glycaemic control and insulin sensitivity in patients with type 2 diabetes mellitusP. de Mol¹, M.J. Fokkert², S.T. de Vries³, E.J.P. de Koning⁴, B.D. Dikkeschei², R.O.B. Gans⁵, C.J. Tack¹, H.J.G. Bilo⁶;¹Internal Medicine, 463, Radboud University Nijmegen Medical Centre,²Clinical Chemistry, Isala Clinics, Zwolle, ³Cardiology, Tjonderschans Hospital, Heerenveen, ⁴Endocrinology and Nephrology, Leiden University Medical Centre, ⁵Internal Medicine, University Medical Centre Groningen,⁶Internal Medicine, Isala Clinics, Zwolle, Netherlands.

Background and aims: Little is known regarding the effects of high altitude trekking in type 2 diabetes mellitus. We investigated the effects of high altitude trekking preceded by exercise training on glycemic control, plasma lipids and insulin sensitivity in patients with type 2 diabetes mellitus.

Materials and methods: Thirteen individuals with complication-free type 2 diabetes mellitus took part in a 13 day expedition to the summit of Mount Toubkal (4167m), Morocco, after six months exercise training. Following baseline maximal exertion ergometer testing, participants received an individualized training advice. During the exercise training and at high altitude, energy expenditure, body weight, fasting glucose, HbA_{1c}, plasma lipids and insulin sensitivity (HOMA-IR) were determined. Additionally, continuous glucose monitoring and acute mountain sickness scores (AMS) were measured at altitude.

Results: Exercise training reduced body weight (-3.5 ± 4.2 kg; $p=0.006$), triglycerides (-0.9 ± 0.8 mmol/L; $p=0.025$), fasting glucose (-0.9 ± 0.9 mmol/L; $p=0.026$) and hypoglycemic medication and increased exercise capacity ($+0.3 \pm 0.3$ W/kg; $p=0.0047$). These changes persisted at the end of the high altitude trekking part. All participants reached the summit at 4167 m. At 3200m altitude, insulin sensitivity was improved compared to values prior to the start of exercise training (3.35 ± 1.85 vs. 1.92 ± 0.85 ; $p=0.011$). Additionally, total cholesterol (-1.2 ± 1.4 mmol/L; $p=0.014$) and LDL-cholesterol (-0.5 ± 0.9 mmol/L; $p=0.012$) were reduced compared to values prior to the start of exercise training. Frequency and duration of hyper- or hypoglycemic episodes (CGMS) remained unchanged at high altitude compared to sea level. At altitude, fasting glucose decreased ($p=0.043$), acute mountain sickness scores remained low and energy expenditure increased ($p<0.01$). (Fig.1).

Conclusion: High altitude trekking preceded by 6 months exercise training can be achieved and improves plasma lipids, insulin sensitivity and glycemic control in patients with complication-free type 2 diabetes.

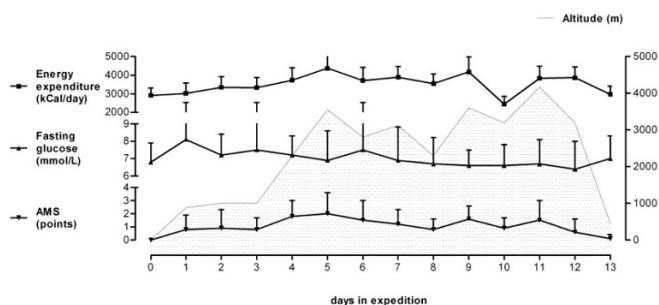


Figure 1: Daily energy expenditure (upper panel), fasting glucose (n=10) (middle panel) and acute mountain sickness score during the 13-day expedition. Altitude presented refers to the highest altitude reached that specific day. (shaded area, right Y-axis) Data are presented as mean \pm SD. Note: Day 0 refers to baseline measurements after completion of the preparation period at sea level. Day 1, 10 and 13 represent resting days.

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Exercise training in patients with type 2 diabetes mellitus induces tissue specific changes in (ectopic) fat contentJ.T. Jonker¹, P. de Mol², S.T. de Vries³, R.L. Widya⁴, H.E. Kan⁴, S. Hammer⁴, R.O.B. Gans⁵, L.D. Dikkeschei³, A. Webb⁴, E.J.P. de Koning⁶, H.J.G. Bilo³, H.J. Lamb⁴;¹Endocrinology and Metabolism, Leiden University Medical Center,²UMCN, Nijmegen, ³Isala Clinics, Zwolle, ⁴Radiology, Leiden UniversityMedical Center, ⁵UMCG, Groningen, ⁶Nephrology, Leiden University

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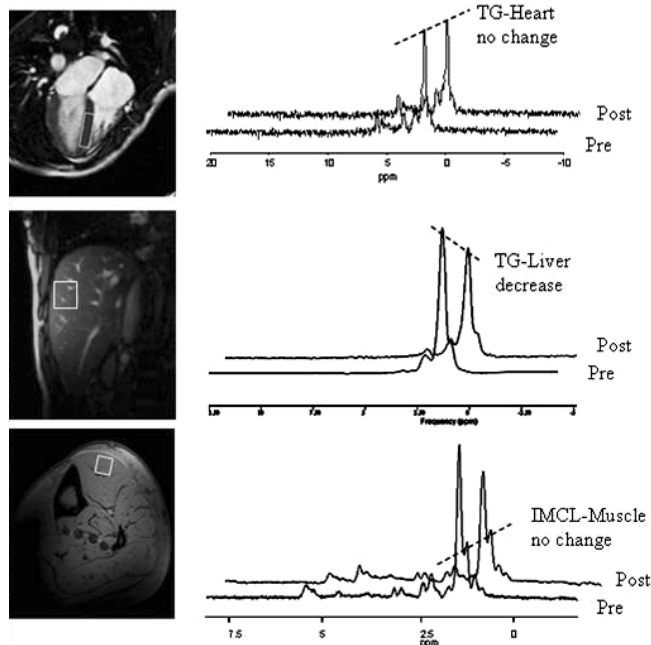
Background and aims: Ectopic fat accumulation in type 2 diabetes is associated with insulin resistance, lipid abnormalities and increased risk for cardiovascular disease. Lifestyle changes are a pillar of therapy. Although changes

in diet have beneficial effects on ectopic fat accumulation, this is less clear for effects of exercise training. Therefore, the purpose of the present study was to assess the effects of a 7-months exercise program on (ectopic) fat accumulation in heart, liver, abdomen, and skeletal muscle.

Materials and methods: We included 12 patients with type 2 diabetes mellitus (mean \pm SEM: age 46 ± 2 years; BMI 28.7 ± 1.2 kg/m²). The training program consisted of 7-months individualized training and ended with climbing the Toubkal mountain (the highest peak in the Atlas Mountains). Before training and again after climbing the mountain, patients were studied. Abdominal fat volume was measured using magnetic resonance (MR) imaging. Myocardial, hepatic and myocellular triglyceride (TG) content were measured using proton MR spectroscopy.

Results: A 7-months exercise program in patients with type 2 diabetes mellitus significantly reduced visceral abdominal fat volume from 348 ± 57 ml (mean \pm SEM) to 219 ± 33 ml ($P<0.01$). Hepatic triglyceride content decreased from $6.8\pm2.3\%$ (mean \pm SEM) to $4.6\pm1.6\%$ ($P<0.01$). Exercise did not change myocardial TG content ($0.61\pm0.13\%$ to $0.60\pm0.13\%$, $P>0.05$). Skeletal muscle intramyocellular lipids/ creatine ratio did not significantly change (3.17 ± 0.67 (mean \pm SEM) at baseline; 4.11 ± 0.76 ($p>0.05$) at follow up).

Conclusion: A 7-month exercise intervention in patients with type 2 diabetes mellitus decreases visceral abdominal fat volume and hepatic TG content. Myocardial and myocellular lipid content remained unchanged. Therefore, exercise intervention in patients with type 2 diabetes mellitus induces tissue-specific changes in ectopic fat distribution.



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Association between physical activity estimated by the international physical activity questionnaire and UKPDS cardiovascular risk score in type 2 diabetes

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Background and aims: Physical activity (PA) is the most effective strategy for the prevention of type 2 diabetes mellitus and an indispensable tool for the treatment of diabetes-associated metabolic abnormalities. However, quantitative analysis of PA in patients with type 2 diabetes is not simple and standardized, and limited data are available on the association between PA and cardiovascular risk in type 2 diabetes. The objective of this study was to correlate the daily energy expenditure from PA, estimated by the International Physical Activity Questionnaire (IPAQ), and the risk of developing cardiovascular disease, calculated by the UKPDS risk engine, in subjects with type 2 diabetes.

Materials and methods: PA-associated energy expenditure, expressed in Metabolic Equivalents (METs), was estimated in 576 type 2 diabetic patients

(324 male, 252 female; mean age, 63 years; body mass index, 29.5 ± 5.5 kg/m²; HbA1c, 7.03 ± 1.2), using the IPAQ. The questionnaire was firstly self-compiled and then revised by an interviewer with the patient. Metabolic and clinical data were obtained from each patient and UKPDS risk score was calculated.

Results: Mean energy expenditure was found to be high in 3.3% (≥ 3000 METs-min/week), moderate in 40.8% (600–3000 METs-min/week), and low in 55.9% of the population (≤ 600 METs-min/week), respectively, according to the IPAQ Guidelines for Data Processing and Analyses. When the population was divided into quartiles of METs, calculated risk for total coronary heart disease (CHD) was found to be 20.72% in the 1st quartile vs. 16.27 in the 4th quartile ($p = 0.051$), fatal CHD risk 15.84% vs. 11.50% ($p < 0.05$), and stroke risk 15.45% vs. 10.47% ($p < 0.05$), respectively.

Conclusion: Evaluation of physical activity with a structured questionnaire correlates with the estimated cardiovascular risk and may contribute to the phenotypic profiling of type 2 diabetic patients.

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Association between physical activity level and diabetic complications among Bangladeshi type 2 diabetic subjects

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Background and aims: Physical activity exerts numerous beneficial health effects, and the evidence favoring a physically active lifestyle in the treatment of chronic diseases is substantial. Physical activity is a cornerstone of type 2 diabetes management, but is often underutilized. Regular physical activity may prevent diabetic complications through beneficial effects on glycemic control, insulin sensitivity, blood pressure, lipid profile, and endothelial function. However, physical activity could also cause adverse effects or patients may not be able to exercise due to complications. No such study has so far been done about the relationship between physical activity and diabetic complications in a Bangladeshi population. The aim of the study was to assess the association of physical activity with the presence of diabetes related complications among Bangladeshi type 2 diabetic subjects.

Materials and methods: The cross-sectional study was conducted in the OPD of BIRDEM, a tertiary diabetic care hospital. A group of 977 subjects were randomly selected and followed up. A structured questionnaire was administered to collect socio-demographic information. Diabetes was diagnosed following the WHO study group criteria. The level of physical activity was categorized into light and moderate. The WHO recommended Asian criteria was used to identify general obesity as healthy weight (BMI 18.5–22.99 kg/m²), overweight (23.0–24.99 kg/m²) and obese (25.0 and above kg/m²). Retinopathy was detected by fundal photography, CKD by serum creatinine and hypertension was diagnosed clinically. Univariate and multivariate generalized linear models were used to assess the associations of physical activity with diabetes related complications.

Results: Out of the 977 subjects investigated, 468 were male and 509 were female (mean \pm SD, age 56 ± 8 years). In the study subjects 74% were engaged in light physical activity and of them 65% subjects were obese. On the other hand 26% were involved in moderate activity and of them 61% subjects were obese. Among the male 387 (82.7%) were engaged in light work and 81 (17.3%) were involved in moderate work. On the other hand 336 (66%) and 173 (34%) female were involved in light and moderate works respectively. On Univariate analysis, light physical activity level showed significant association with all the three diabetes related complications ($p < 0.001$). And in the middle age group (46–60 yrs), the risk of developing hypertension was 2 times higher in light compared to moderate activity group. For CKD the corresponding risk is 3 times higher. Multivariate analysis showed low level of physical activity was strongly associated with complications like retinopathy [$p = 0.00$, (95% CI, 0.02–0.08)] & hypertension [$p = 0.01$, (95% CI, 0.34–0.86)] in the females. Low level of physical activity was also found to be strongly associated with retinopathy [$p < 0.001$, (95% CI, 0.01–0.11)] in the male group.

Conclusion: A large number of urban of Bangladeshi population are involved only light physical activity which is leading to obesity and it seems to have a strong association with diabetes related complications in this population.

Supported by: BADAS, NOMA

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Strength training and insulin sensitivity in patients with end-stage renal disease undergoing dialysis

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Background and aims: Insulin resistance is common in patients undergoing dialysis and has associations with protein degradation and cardiovascular disease. The clinical implications are a loss of muscle protein as well as an increased morbidity and mortality. The aims of the present study were to investigate the effects of high-load strength training on insulin sensitivity in such patients, and to measure any benefits of protein supplementation.

Materials and methods: 23 patients (aged 52 ± 3 ; mean \pm SE: 10/13 male/female; BMI 23.9 ± 0.9 kg/m²) on hemodialysis ($n = 19$) or peritoneal dialysis ($n = 4$) were recruited to a control period of 16 weeks before participating in 16 weeks of strength training. The training consisted of 5 minutes of warm-up and up to 5 sets of three obligatory exercises three times a week: leg press, leg extension and leg curl. The period of rest between each set was of 60–90 seconds in duration. During the intervention period the load was increased, and the repetition maximum decreased from 15 to 6. The progression in training was adjusted according to changes in strength during the intervention period. The patients received an isocaloric drink immediately after each training session, which was randomly assigned to provide either a protein or non-protein source. Insulin sensitivity was tested using the 2-hour OGTT after an over-night fast. Plasma glucose and insulin were tested at -30 min; -15 min; 0 min; 30 min; 60 min; 90 min; and 120 min.

Results: At baseline the glucose tolerance was normal for 9 patients, impaired in 11 patients, and 3 patients had type 2 diabetes. Body weight was unchanged during the control period and increased significantly after the intervention (from 72.8 ± 3.7 to 73.9 ± 3.8 kg, $p = 0.05$; Wilcoxon Test). Fasting, and 2-hour blood glucose measurements remained unchanged during the control and the training periods (during training, fasting from 5.3 ± 0.1 to 5.5 ± 0.2 mmol/l, and 2-hour from 8.9 ± 0.6 to 8.5 ± 0.7 mmol/l). Likewise, insulin values remained unchanged during the control period, but were significantly decreased after training (fasting from 65 ± 8 to 53 ± 7 pmol/l, $p = 0.01$, and 2-hour from 423 ± 70 to 326 ± 52 pmol/l, $p < 0.05$). The effects on fasting insulin were found to be associated with baseline fasting insulin values ($r = 0.654$, $p = 0.001$). In parallel with the decrease of insulin, significant improvements were seen in muscle power (33 \pm 5% increase, $p < 0.001$) and in muscle strength, physical function, and quality of life (data not shown). Finally, an initial unadjusted analysis did not reveal any benefits of combining the training period with supplementing protein intake.

Conclusion: This simple high-load strength training program was found to be associated with a significant improvement in insulin sensitivity in patients undergoing dialysis. The most relevant exercise method in the treatment of insulin resistance has been considered to be aerobic training. However, the findings of the present study would suggest that strength training is also a very relevant method.

Supported by: Nutricia

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Impact of body weight on exercise capacity and changes in metabolic parameters during spiroergometry in children and adolescents

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Background and aims: Physical fitness is supposed to be reduced in obese children, most likely due to reduced physical activity. Regular exercise is known to improve metabolic parameters such as insulin resistance. Therefore the aim of our study was to compare exercise capacity and acute exercise-related changes in metabolic parameters between lean and obese children.

Materials and methods: We evaluated 65 children (34 girls, 31 boys) aged 14.5 ± 2.9 years stratified into lean (BMI SDS 0.16 ± 0.55 , $n = 33$) and obese (BMI SDS 2.54 ± 0.54 , $n = 32$) by spiroergometry. Children were asked to cycle to maximum exhaustion applying a two minute step programme of 0.5W/body weight adapted to the 50th centile of height. Exercise parameters were standardized to age and sex references and are given in %. Blood samples were drawn at baseline and after the test.

Results: Physical performance, assessed by the highest intensity in Watt achieved, was significantly better in lean compared to obese children

(118.6 ± 5.18 % vs. 81.03 ± 3.62 %; $p < 0.0001$). Similarly, maximum oxygen uptake ($\text{VO}_{2\text{max}}$), which is independent of training condition and motivation, was significantly reduced in obese children (81.27 ± 3.23 vs. 114.67 ± 4.27 %; $p < 0.0001$). At maximum effort, the respiratory quotient (RQ) was 1.105 ± 0.01 in the lean compared to 1.045 ± 0.01 in the obese children, hence indicating that obese children stopped cycling before the maximum physical capacity was reached ($p < 0.0001$). This observation was confirmed by the increase in lactate levels that were almost doubled in the lean compared to the obese group (5.74 ± 0.54 vs. 2.89 ± 0.46 ; $p < 0.0001$). The anaerobic threshold (AT) is indicating how quickly the metabolism switches to an anaerobic state. After the AT was reached the obese group terminated cycling earlier (3.53 ± 0.39 vs. 5.82 ± 0.57 ; $p = 0.0025$). Basal glucose (5.41 ± 0.19 vs. 4.52 ± 0.20 ; $p = 0.0025$) and insulin levels (463.20 ± 105.1 vs. 181.61 ± 33.63 ; $p = 0.010$) were significantly higher in the obese subjects. During exercise, insulin ($p = 0.0003$) and glucose ($p = 0.05$) levels were reduced to a greater extent in obese individuals (insulin -146.58 ± 74.75 ; glucose -0.22 ± 0.16) compared to -29.82 ± 31.22 (insulin) and 0.30 ± 0.21 (glucose) in the lean group. The levels of free fatty acids and triglycerides showed a slight increase in the lean subjects and a reduction in the obese cohort after exercise (not significant). Finally, blood pressure and heart rate returned more rapidly to initial values post exercise during the recovery phase in lean compared to obese children ($p = 0.009$).

Conclusion: Obese children showed a significant reduced exercise capacity with lower physical performance. Interestingly, we demonstrated a significant and immediate improvement in the metabolic profile by acute exercise. In summary our results further underline the beneficial effects of increased physical activity on metabolic and cardiovascular risk in obese children.

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PS 041 Alternative treatments for insulin resistance

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A natural compound suppresses hepatic gluconeogenesis and lipid synthesis by activation of AMPK

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Background and aims: YL01, a natural compound has been identified to activate AMPK and increase glucose uptake and free fatty acid oxidation in L6 myotubes in our previous study. In the present study, the effect of YL01 on hepatic gluconeogenesis and lipid synthesis was evaluated and its possible mechanisms were explored.

Materials and methods: Measurement of glucose production was performed in primary hepatocytes under glucagon stimulated state to examine the effect of YL01 on hepatic gluconeogenesis. Fatty acid and sterol synthesis in primary hepatocytes were assayed by measuring [^{14}C] acetate incorporation into lipids. Activation of YL01 on the AMPK signaling pathway was investigated by western blot analysis. Pyruvate tolerance test was performed to evaluate the in vivo effect of YL01 on gluconeogenesis in C57 mice.

Results: YL01 dose dependently inhibited glucagon stimulated gluconeogenesis and insulin induced fatty acid and sterol synthesis in primary hepatocytes. $10 \mu\text{M}$ YL01 reduced gluconeogenesis, fatty acid and sterol synthesis by 18.7%, 26.8% and 40.2%, respectively. YL01 significantly increased AMPK and ACC phosphorylation in primary hepatocytes in a dose dependent manner. The YL01 suppression of hepatic gluconeogenesis and lipid synthesis was fully blocked by the pretreatment with compound C, an AMPK inhibitor. Single oral administration of YL01 at dose of 300mg/kg in C57 mice significantly increased AMPK and ACC phosphorylation in liver. Moreover, administration of YL01 caused significant reduction in blood glucose levels after pyruvate loading, suggesting the inhibitory role of YL01 on gluconeogenesis in vivo.

Conclusions: Our results demonstrated that YL01 inhibited hepatic gluconeogenesis and lipid synthesis by activation of AMPK, which suggested that it might be a therapeutic candidate for the treatment of T2DM and metabolic syndrome.

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Impact of Lactobacillus casei Shirota supplementation on insulin sensitivity and beta cell function in subjects with metabolic syndrome

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Background and aims: Based on animal studies, intake of probiotic bacteria was suggested to improve glucose homeostasis by reducing endotoxaemia and inflammation. We hypothesized that Lactobacillus casei Shirota (LcS) supplementation might have an impact on glucose tolerance and indices of insulin sensitivity and β -cell function in subjects with the metabolic syndrome and insulin resistance.

Materials and methods: In a single-centre, prospective, randomized-controlled pilot study, 30 subjects with metabolic syndrome received either Lactobacillus casei Shirota 3 times daily for 12 weeks or served as controls with standard medical therapy. A 75-g oral glucose tolerance test (oGTT) was performed and blood samples were collected to determine plasma glucose, serum insulin and serum c-peptide. Insulin sensitivity was assessed by homeostasis model assessment (HOMA-IR), insulin sensitivity index (ISI), Matsuda-Index (ISO-GTT) and QUICK-index. Beta-cell function was quantified as the ratio of incremental insulin to glucose responses over the first 30 min during the OGTT, the HOMA- β index and by 1st and 2nd phase insulin secretion indices.

Results: Insulin sensitivity index (ISI) was significantly improved after 3 months of probiotic supplementation (0.058 ± 0.021 vs. 0.038 ± 0.025 , $p < 0.01$) but not significantly different to the control group. Likewise the ratio of incremental insulin to incremental glucose responses over the first 30 minutes during the OGTT significantly decreases during the treatment in the LcS group (73.2 ± 64.6 to 47.7 ± 44.2 , $p < 0.05$), but again without a significant difference to the control group. No improvements were seen in further indices calculated.

Conclusion: In conclusion, intake of LcS for 12 weeks in subjects with metabolic syndrome did not clearly affect insulin sensitivity or β -cell function in this pilot trial.

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S-nitrosothiols as potential pharmacological targets on the treatment of insulin resistance

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Background and aims: It has been shown in fasted Wistar rats that co-administration of NO and GSH, via portal vein, was able to promote an increase in insulin sensitivity of 125.3±18.6%. From this result it was proposed that increasing hepatic glutathione and nitric oxide levels forms nitrosothiols (RSNOs), which are release into the bloodstream, acting on extra-hepatic tissues by increasing insulin sensitivity. An endocrine function of RSNOs has already been established by other authors, hinting to the possibility the previous hypothesis that the synthesized RSNOs in the liver will act on periphery. These observations highlight these molecules as potential pharmacological drugs in the treatment of insulin resistance, therefore our hypothesis is that co-administration of NO and GSH or administration of RSNOs is able to overcome the insulin resistance induced by a high-sucrose diet.

Materials and methods: 12 week old Wistar rats (control vs. high sucrose) were used. The high-sucrose group consisted in animals fed with a high-sucrose diet (35% (w/v)) for a 4 week period (8-12 weeks of age). Insulin sensitivity was assessed by a rapid euglycaemic insulin sensitivity test. Insulin sensitivity was determined in the fasted state and i) after a standard meal, ii) after GSH (GSH-E: 1 or 2 mmol/kg) + NO (SIN-1: 50 or 100 μ mol/kg) administration into the portal vein or an S-nitrosothiol (S-nitrosoglutathione-GSNO: 50 μ mol/kg or 100 μ mol/kg) intravenous administration. Insulin and C-peptide levels were determined by ELISA Kit.

Results: Insulin sensitivity in the control animals increased 89.1±15.3% after a meal, but this potentiation was not observed in the high sucrose diet (10.3±8.9%, **, $p<0.01$) GSH (1mmol/kg) and NO (50 μ mol/kg) administered in fasted animals, directly to the portal vein, increased insulin sensitivity by 125.3±18.6%. On the other hand the administration of both drugs to the high-sucrose model was unable to increase insulin sensitivity even when we doubled the dose. RSNOs administration increased insulin sensitivity in both controls and high sucrose groups. However, full potentiation (100.1±38.5%) was only observed in the high sucrose diet when we doubled the dose of GSNO (100 μ mol/kg). Plasma C-peptide and insulin levels remained unchanged after GSNO administration which indicates that this drug is not affecting insulin secretion or insulin clearance.

Conclusion: The results presented herein allow us to conclude that in the high sucrose animal model the insulin resistance observed was not overcome by administration to the liver of GSH and NO, indicating inability of the liver to synthesize RSNOs. On the other hand, GSNO administered to the periphery was able to overcome the insulin resistance observed in this animal model, and this mechanism seems to be independent of insulin secretion. Our *in vivo* results showed for the first time that S-nitrosothiols induces increases in insulin sensitivity, indicating these drugs as potential pharmacological tools in the treatment of peripheral insulin resistance.

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Red grape polyphenols prevent deleterious metabolic effects induced by fructose

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Background and aims: Studies have shown that dietary fructose induces dyslipidemia and insulin resistance. In this work we looked at whether the insulin resistance induced by fructose was associated with oxidative stress. We also evaluated the effects of a natural red grape extract on the prevention of metabolic abnormalities caused by fructose.

Materials and methods: 35 overweight and obese 1st degree relatives of type 2 diabetic subjects [20 women / 15 men ; BMI 29.1± 2.2 kg/m² (mean ± SD) ; age 49.2 ± 8.4 years] were randomized in a double blind study between a grape extract (GE) group (2g/day) and a placebo (P) group for a period of 9 weeks. During the last week, both groups received a fructose load (3g/kg lean body mass/day) to be consumed in aqueous form during the 3 major daily meals. Our volunteers were studied before and after the week of fructose overload.

Results: Body weights remained stable whereas fasting plasma triglyceride concentrations increased similarly in both groups (GE 36%, P 31%). However, glucose disposal measured by the hyperinsulinemic euglycemic clamp was decreased in the P group (7.37 ± 2.36 vs 6.64 ± 2.38 mg/kg/min, $p=0.01$) but not in the GE group (6.72 ± 3.09 vs 6.50 ± 1.99 mg/kg/min, NS). Overnight urinary F2-isoprostanes (a marker of oxidative stress) were increased after fructose in the P group (475 ± 165 vs 554 ± 183 pmol/mmol creatinine, $p=0.01$) whereas they were decreased in the GE group (513 ± 187 vs 473 ± 148 pmol/mmol creatinine, $p=0.05$). For all subjects, insulin sensitivity before fructose was inversely correlated with urinary F2-isoprostane levels ($r=-0.46$, $p<0.01$) and insulin sensitivity fluctuations after fructose were negatively correlated with changes in urinary F2-isoprostane levels ($r=-0.32$, $p=0.05$).

Conclusion: These data suggest that fructose induced insulin resistance might in part be secondary to oxidative stress and can be prevented by a polyphenolic rich red grape extract in a population of overweight/obese adults. Therefore our results help better understand the mechanistics behind insulin resistance in order to counterattack the ever growing worldwide diabetes epidemic.

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Mechanism of action of VVP808: a novel insulin sensitising agent

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Background and aims: We used gene expression signature technology to identify a family of compounds with insulin sensitising activity. The aim of these studies was to characterise the effects of these compounds in animal models of insulin resistance and diabetes, and to investigate the molecular mechanism of action of these compounds.

Materials and methods: The VVP800 family of compounds was tested in diet induced obese (DIO) mice, db/db mice and STZ rats. The lead compound, VVP808, was administered by single daily oral gavage to animals for 12-40 days at a range of doses, and efficacy was assessed by measuring body weight, blood glucose, insulin and HbA1c concentrations, and by conducting hyperinsulinemic-euglycemic clamps.

Results: VVP808 reduced fasting blood glucose concentration ($p=0.00004$) and HbA1c levels ($p=0.04$) in db/db mice, and improved glucose tolerance in diet-induced obese (DIO) mice ($p<0.05$). VVP808 also reduced body weight by up to 14% ($p<0.01$) and epididymal fat pad weight by up to 48% ($p=0.01$) in DIO mice. In STZ diabetic rats, VVP808 (50 mg/kg/d for 12 days) had no effect on fasting blood glucose concentration. However, VVP808 significantly enhanced the glucose-lowering effects of exogenous insulin (0.5U/kg) in an insulin tolerance test (by 2.2-fold after 30 min ($p=0.045$) and by 2.4-fold after 60 min ($p=0.038$)). Data from hyperinsulinemic-euglycemic clamp studies

in DIO mice showed that treatment with VVP808 (50 mg/kg/d for 14 days) increased the glucose infusion rate by 27% ($p=0.005$), and this was associated with a 23% decrease in endogenous glucose production ($p=0.04$). We are currently conducting a range of studies to identify the molecular mechanism of action of VVP808 including ligand capture affinity chromatography studies using analogues of VVP808 and protein lysates extracted from livers of mice treated with VVP808, and microarray pathway analysis studies in tissues of animals treated with VVP808.

Conclusion: VVP808 is a novel insulin sensitising agent that acts primarily on the liver to suppress endogenous glucose production.

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C-peptide enhances *in vitro* and *in vivo* insulin action

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Background and aims: *In vitro* studies have shown that C-peptide interacts with hexameric insulin and facilitates its disaggregation into the physiologically active monomeric form. With the aim to further investigate this effect under *in vivo* conditions we have employed the euglycemic hyperinsulinemic clamp technique in rats and examined skeletal muscle and liver biopsy material after subcutaneous administration of both insulin and C-peptide either in a mixture or as separate injections.

Material and methods: Male Wistar rats (age 6 weeks, 145–165g) were used. Regular insulin (I) (1.2 U/kg) was injected subcutaneously (s.c.) together with 29 nmol/kg rat C-peptide (C). I and C were administered either as a physical mixture or separately into two s.c. depots. Rats were awake and allowed to move freely within a large cage during the study. During a 60 min baseline period the ambient glucose level was established and subsequently clamped ± 0.3 mM by frequent blood glucose analyses and adjustment of a variable i.v. glucose infusion for approximately 2 h. In some experiments the study was terminated at 30 min post I and C injection, anaesthesia commenced and biopsy samples were obtained from liver and hindlimb muscles and frozen immediately. Akt and GSK3 phosphorylation in liver and muscle were examined using Western blot technique. Finally, Na^+ , K^+ -ATPase activity was assessed as ouabain-sensitive $^{86}\text{Rb}^+$ uptake by L6 myotubes before and after incubation with insulin with or without the presence of C-peptide.

Results: Subcutaneous injection of a mixture of I and C resulted in a 30% greater ($P<0.01$) and 15% ($P<0.05$) longer stimulation of whole body glucose utilization than observed after injection of equimolar amounts of I and C into separate depots. Plasma concentrations of insulin were higher 30 min after injection of combined I and C (15.2 ± 2.8 ng/mL) compared to after separate injections of I and C (10.1 ± 1.3 ng/mL, $P<0.03$). Insulin-stimulated phosphorylation of Akt/PKB in liver but not muscle increased 32% more ($P<0.06$) after injection of I and C in mixture compared to separate injections. Likewise, phosphorylation of GSK3 was augmented by 50% ($P<0.05$) following administration of I and C mixture compared to separate injections. Exposure of L6 myotubes to 1 nM C-peptide for 15 min stimulated ouabain-sensitive $^{86}\text{Rb}^+$ uptake by 31% ($P<0.05$), while 1 nM of insulin had no effect. Stimulation of myotubes by pre-mixed equimolar C-peptide and insulin (1 nM) elicited 20% additional increase in ouabain-sensitive $^{86}\text{Rb}^+$ uptake ($P<0.05$) in comparison to the effect 1 nM C-peptide alone. This additive effect was lost when insulin and C-peptide were added separately to myotubes.

Conclusion: Subcutaneous co-administration of insulin and C-peptide results in increased insulin availability and augmented insulin effects both at the level of glucose uptake and intracellular signaling and enzyme activation. These effects may be attributed to augmented disaggregation of hexameric insulin into its physiologically active monomeric form.

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Isomaltulose tightens pre-exercise glycaemia and produces similar run performance in type 1 diabetes

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Background and aims: Recent literature has shown that pre-exercise ingestion of the low glycaemic index (GI) CHO isomaltulose can increase blood glucose (BG) concentrations less before exercise and protect against hypoglycaemia following exercise through lesser suppression of lipid oxidation compared to a high GI CHO in type 1 diabetes individuals (T1DM). However, it is not known what effect low GI CHO consumption has on exercise performance. This study compared the alterations to metabolic and performance responses to aerobic running following ingestion of a low- and high-GI CHO. **Materials and methods:** With ethical approval, 7 individuals (34 ± 5 years, 70 ± 2 kg, HbA_{1c} 76.6 ± 6.5 mmol.mol⁻¹) completed this study. After preliminary testing, participants attended the laboratory twice and were provided with 0.6 g.kg^{-1} body mass of either dextrose (DEX) or isomaltulose (ISO, Palatinose®) immediately after they had administered a 50% reduced dose of their rapid-acting insulin. After 2-h rest participants completed a 26 minute discontinuous incremental treadmill protocol (4 min running; 1.5 min rest at 31, 41, 53, 69 and 80% VO_2max) before completing a 10 min run on a non-motorised treadmill where participants were instructed to complete as much distance as possible in this time. Capillary blood samples were obtained prior, during and after exercise and were analysed immediately for BG and lactate (GEM, Instrumentation Laboratories). Data was analysed by repeated-measures ANOVA with post-hoc testing where appropriate and expressed as mean \pm SEM. Statistical significance was accepted at $P<0.05$.

Results: Resting blood glucose concentrations were similar between conditions. Relative peak BG was lower after ingestion of ISO compared to DEX (ISO 5.6 ± 0.4 vs. DEX 10.3 ± 0.7 mmol.l⁻¹, $P<0.05$), and the time to reach this peak value took longer in ISO (86 ± 8 min) compared to DEX (64 ± 4 min) ($P<0.05$). Changes in mean BG throughout the 2 h rest period were lower in ISO than DEX (ISO 4.6 ± 0.4 vs. 7.8 ± 0.8 mmol.l⁻¹, $P<0.05$), as were both the rest incremental area under the curve (IAUC) (ISO 483.9 ± 40.5 vs. DEX 847.6 ± 72.4 mmol.min.l⁻¹, $P<0.05$) and total IAUC (ISO 627.1 ± 67.8 vs. DEX 1057.9 ± 119.8 mmol.min.l⁻¹, $P<0.05$) over the length of the trial. BG reductions throughout the submaximal exercise were similar between both CHO (ISO -1.9 ± 0.5 vs. DEX -1.0 ± 0.5 mmol.l⁻¹, $P>0.05$) as were the BG changes in the 10 min run (ISO 0.7 ± 0.4 vs. DEX 0.7 ± 0.3 mmol.l⁻¹, $P>0.05$). Resting blood lactate concentrations were similar between conditions however, following ISO ingestion blood lactate was higher over the 2 h rest period ($P<0.05$). There were no differences in lactate concentrations between CHO once exercise began. Distance covered during the 10 min run was similar between conditions (ISO 1.14 ± 0.09 vs. DEX 1.15 ± 0.09 km, $P>0.05$).

Conclusion: Pre-exercise ISO ingestion of 0.6 g.kg^{-1} BM with 50% reduction of rapid-acting insulin caused a smaller rise in blood glucose concentration before exercise and resulted in a similar performance during a 10 minute run. Pre-exercise consumption of ISO is an effective CHO for those exercising T1DM individuals wishing to improve glycaemic control without loss of exercise performance.

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Effects of vitamin D₃ on beta cell function and insulin resistance in children at increased risk of type 1 diabetes

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Background and aims: Studies in subjects at risk for type 2 diabetes as well as animal studies suggest that vitamin D affects β -cell function and insulin resistance. The relationship of Vitamin D levels especially of 1,25(OH)₂ vitamin D₃ (the active form of vitamin D) and β -cell function in healthy children has never been studied. Vitamin D status and supplementation of Vitamin D during early childhood has been associated with type 1 diabetes (T1D) risk. A priori β -cell function and insulin resistance may also play a role in the development of type 1 diabetes. The aim of our study was to determine whether

vitamin D3 status is associated with β -cell function and insulin resistance in children at increased risk for T1D.

Materials and methods: 1,25(OH)₂ vitamin D3 (vitamin D3) was measured in fasting serum samples taken from 156 children participating in the TEENDIAB Study, a prospective cohort study that follows children aged 8–12 years with a family history of T1D during puberty to the age of 18 years. Each child underwent an intravenous glucose tolerance test (median age 10.5 years). Fasting and stimulated insulin and C-peptide was determined at time points 1, 3, 5, 7, 10 min (after glucose load) using an automated immunoassay analyzer (AIA 360; Tosoh, San Francisco, CA). First phase insulin response (FPIR) was calculated as the sum of the serum insulin concentrations at 1 and 3 minutes after glucose load. C-peptide and insulin response during IVGTT were evaluated as area under the curve (AUC) by using the TAI's formula. Insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR).

Results: Median observed vitamin D3 serum concentrations were 38 ng/l (IQR: 32–44 ng/l). Vitamin D3 serum concentrations were positively associated with fasting insulin ($p=0.032$), fasting C-peptide levels ($p=0.007$) and were correlated with HOMA-IR ($p=0.042$). Furthermore when serum vitamin D3 levels were categorized into tertiles (first tertile: 17–34 ng/l, second tertile: 35–42 ng/l, third tertile: 43–61 ng/l) those children with the highest vitamin D3 concentration (third tertile) had a significantly higher fasting C-peptide level compared to the children in the second or first tertile (median C-peptide 1.5 ng/ml vs 1.4 ng/ml vs 1.1 ng/ml, $p=0.006$). These associations remained significant after adjusting for age and gender ($p=0.022$). Vitamin D3 serum levels were not associated with stimulated insulin secretion (FPIR ($p=0.4$), AUC insulin ($p=0.4$) and AUC C-peptide ($p=0.2$)).

Conclusion: This is the first study showing an association of the active form of vitamin D3 with β -cell function and insulin resistance in children aged 8–12 years at increased risk for T1D.

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PS 042 Metabolic emerging biomarkers

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Elevated plasma levels of SPARC in patients with newly diagnosed type 2 diabetes mellitus

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Background and aims: SPARC (secreted protein acidic and rich in cysteine), firstly identified in bone, is also known as osteonectin and BM-40. Although it is expressed in most tissues, adipose tissue cells are the major source of circulating SPARC in subjects with obesity. As an extracellular regulatory macromolecule regulating cell-matrix interaction, SPARC is a multifunctional protein involved in osteogenesis, angiogenesis, wound healing, tumorigenesis, and fibrosis in kidney and liver. Recently, SPARC is suggested as a key player in the pathogenesis of obesity and type 2 diabetes mellitus. However, the pathophysiologic roles of SPARC in glucose metabolism are not well understood. In the present study, plasma levels of SPARC in normal, impaired glucose regulation (IGR) and T2MD subjects were evaluated to better understand its clinical importance. The relationships between circulating plasma SPARC levels and body mass index (BMI), blood lipids, blood glucose, plasma insulin, HOMA-IR, and other factors, were also examined.

Materials and methods: Fifty-four newly diagnosed type 2 diabetic subjects (T2MD), fifty-three subjects with impaired glucose regulation (IGR), and fifty-three normal subjects were enrolled in this study. Plasma SPARC levels were measured with an Enzyme-Linked Immunosorbent Assay (ELISA) under overnight fasting conditions. The relationships between plasma SPARC and several metabolic factors, such as body mass index (BMI), blood lipids, blood glucose, plasma insulin levels and other factors were also assessed.

Results: SPARC levels were higher in subjects with T2MD compared with IGR and control subjects [(16.7 \pm 6.9 vs. 14.0 \pm 8.0) μ mol/L, $P<0.05$ and (16.7 \pm 6.9 vs. 11.7 \pm 4.4) μ mol/L, $P<0.01$]. However, there was no difference in plasma SPARC levels between subjects with IGR and the controls. Plasma SPARC levels were correlated positively with BMI, the percentage of fat (FAT%), Triglyceride (TG), Fasting plasma insulin (FINS), 2h plasma insulin after glucose overload (2hINS), and HOMA-insulin resistance index (HOMA-IR) by simple regression analysis. TG and HOMA-IR were independent related factors influencing plasma SPARC levels by multiple regression analysis ($Y=1.002X_{TG}+1.233X_{HOMA-IR}+9.07$).

Conclusion: Our results showed an increased SPARC level in obese and T2DM subjects suggesting that SPARC may play a role in pathogenesis of both obesity and diabetes. However, further studies need to clarify the biological mechanisms involving SPARC in the pathogenesis of T2DM.

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Suppressive effect of hyperinsulinaemia on serum IL-18 concentration in young, healthy subjects

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Background and aims: IL-18 is a proinflammatory and proatherogenic cytokine which is associated with obesity, insulin resistance and cardiovascular disease. It is supposed that insulin might have anti-inflammatory action, however, its effect on IL-18 is unclear. The aim of the present study was to estimate serum IL-18 concentration in young healthy population, its regulation by hyperinsulinemia and relationship with insulin sensitivity and glucose and lipid oxidation.

Materials and methods: We studied 36 healthy male subjects (mean age, 24.50 \pm 2.67; mean BMI, 25.77 \pm 3.70 kg/m²). Serum IL-18 concentration was measured before and after 2 hour euglycemic hyperinsulinemic clamp. In 18 subjects, clamp was prolonged to 6 hours and at the end an additional measurement of serum IL-18 was taken. RQ and glucose and lipid oxidation

(LOx) were assessed with indirect calorimetry in the baseline state and every 2 hours of the clamp.

Results: Hyperinsulinemia decreased serum IL-18, this effect was present in both 2 and 6 hours of the clamp (both $p < 0.001$). Additionally, serum IL-18 slightly decreased from 2 to 6 hour ($p = 0.044$). Both 2 hour and 6 hour serum IL-18 values were positively related to plasma NEFA in the respective time-points ($r = 0.43$, $p = 0.033$ and $r = 0.52$, $p = 0.028$). Both 2 hour and 6 hour serum IL-18 were also negatively related to RQ ($r = -0.40$, $p = 0.018$ and $r = -0.54$, $p = 0.02$) and positively to LOx ($r = 0.46$, $p = 0.006$ and $r = 0.66$, $p = 0.004$) in the respective time-points. Six-hour IL-18 value was negatively related to insulin sensitivity calculated for the 6th hour of the clamp ($r = -0.53$, $p = 0.025$), serum adiponectin in the 6th hour of the clamp ($r = -0.54$, $p = 0.02$) and positively to the body weight ($r = 0.49$, $p = 0.038$). Additionally, the change in serum IL-18 in response to insulin was inversely related to the white blood cell count ($r = -0.52$, $p = 0.027$) and the neutrophil cell count ($r = -0.56$, $p = 0.016$), i.e., the higher the cell count, the lower the decrease in IL-18.

Conclusion: Our data show that serum IL-18 is negatively regulated by hyperinsulinemia, suggesting anti-inflammatory effect of insulin. IL-18 is related to decreased insulin sensitivity mainly through its association with lipid oxidation.

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Maternal fasting visfatin change and fasting active amylin change predict maternal HOMAR change and weight change respectively during pregnancy

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Background and aims: The increase of maternal weight and insulin resistance during pregnancy are risk factors for gestational diabetes. Adipocytokines and pancreatic hormones affect insulin resistance, glucose balance and food intake. This study looks for predictors among changes of adipocytokines, pancreatic hormones, insulin resistance indices and maternal weight during pregnancy.

Materials and methods: 100 healthy pregnant Caucasian women, aged 28.2 ± 3.8 (mean \pm SD) years, body mass index 24 ± 2.6 kg/m², were randomly seen during each trimester. Each visit they had anthropometrics, fasting plasma sampling for pancreatic hormones (glucagon, active amylin, pancreatic polypeptide-PP) and adipocytokines (adiponectin, visfatin, leptin) and a 75g oral glucose tolerance test (OGTT) for glucose and insulin at 0,15,30,60,90,120 min. Five women developed gestational diabetes (based on WHO criteria) and were separated. OGTT derived indices of carbohydrate metabolism such as insulin resistance (HOMAR), insulin sensitivity (ISI), predicted indexes of first phase and second phase of insulin secretion (1st PHIS, 2nd PHIS) were calculated.

Results: Maternal weight increased significantly during all trimesters of pregnancy ($p < 0.05$). Serum visfatin increased significantly from the 2nd to the 3rd trimester ($p = 0.05$). HOMAR index increased significantly in the 3rd compared to the 1st and 2nd trimester ($p < 0.05$) while the ISI index decreased significantly in the 3rd compared to the 1st and 2nd trimester ($p < 0.05$). At 1st trimester visfatin correlated positively with weight ($p = 0.04$, $r = 0.73$). Leptin correlated positively with weight ($p = 0.0001$, $r = 0.85$), 1st PHIS ($p = 0.004$, $r = 0.65$), and 2nd PHIS ($p = 0.008$, $r = 0.57$) and negatively with ISI ($p = 0.007$, $r = -0.59$). Active amylin correlated positively with 2nd PHIS ($p = 0.04$, $r = 0.42$) negatively with HOMAR ($p = 0.004$, $r = -0.69$). At 2nd trimester glucagon correlated negatively with HOMAR ($p = 0.04$, $r = -0.45$) and adiponectin ($p = 0.03$, $r = -0.93$). Active amylin correlated negatively with glucagon ($p = 0.04$, $r = -0.45$). The longitudinal regression model taking in consideration all three trimesters revealed maternal fasting visfatin change as the best positive and negative predictor of insulin resistance (HOMAR) ($p = 0.0002$, t -value = 4.48) and sensitivity (ISI) ($p = 0.002$, t -value = -3.65) changes, respectively, among changes of glucagon, PP, active amylin, leptin, and adiponectin levels. The longitudinal regression model taking in consideration all three trimesters revealed maternal fasting active amylin change as the best negative predictor ($p = 0.02$, t -value = -2.41) of maternal weight change among changes of glucagon, PP, visfatin, leptin, and adiponectin levels.

Conclusion: The present study shows the impact of maternal fasting active amylin change (anorectic factor) into maternal weight change and of maternal fasting visfatin change (insulinomimetic action) into insulin resistance

change during normal pregnancy. Further studies are needed to investigate the importance of the above predictors into gestational diabetes as early biomarkers of HOMAR change and weight change

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Cathepsin K predicts glycaemic control and beta cell function in impaired glucose tolerant men: a new link between skeleton and energy homeostasis?

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Background and aims: Cathepsin K (Cat K) is a cysteine protease that have been shown to participate in bone resorption, adipogenesis and energy homeostasis. Inhibition or deficiency of Cat K reduced insulin and glucose levels and inhibited body weight gain in mice. Only few clinical data are available about the metabolic role of Cat K in humans, and these are contradictory. Our aim was to compare Cat K levels in normal and impaired glucose tolerant men, to assess its relationship to energy homeostasis, insulin and glucose metabolism.

Materials and methods: 53 healthy and glucose intolerant male subjects (aged 43.3 ± 13.1 years) were examined who were previously non-treated for diabetes. OGTT, ivGTT and hyperinsulinemic euglycemic clamp were done to assess glucose tolerance, insulin secretion and sensitivity. Full metabolic profiling, lipoproteins, adipocytokines, serum Cat K, osteocalcin (OCN), osteoprotegerin (OPG) and nuclear factor κ B ligand (RANKL) were measured. Body composition mass was determined by DEXA.

Results: Based on the OGTT, 21 subjects were normal (NGT) and 32 subjects glucose intolerant (GI = IFG/IGT or 2DM). Serum Cat K levels were somewhat higher in the NGT than in the GI group (11.6 ± 8.2 vs. 7.97 ± 4.76 pmol/l, $p = 0.058$). Serum Cat K levels showed strong positive bivariate correlation with indicators of the 1st phase insulin secretion of ivGTT, i.e. acute insulin response (AIR, $r = 0.38$, $p = 0.01$) and insulinogenic index (IGI, $r = 0.41$, $p = 0.004$), negative correlation with fasting glucose levels ($r = -0.28$, $p = 0.05$), HbA1c ($r = -0.36$, $p = 0.01$) and fasting free fatty acid levels ($r = -0.33$, $p = 0.02$). These correlations were unaffected if data were adjusted with age, body mass index (BMI) and body fat percent (BFP). If the two groups were assessed separately this correlation stayed significant only in the GI group. No correlations were observed between serum Cat K levels and clamp measured muscle glucose utilization, BMI or BFP. With feature selection (variable ranking) analysis serum Cat K level was ranked as 10.5/67.7 (average merit 0.051 ± 0.02) to determine AIR, ranked even higher than ivGTT or OGTT glucose or HbA1c% levels. Similar results were found for IGI.

Conclusion: We found that serum Cat K levels predict β cell function in GI men independent of body composition or insulin sensitivity. This finding differs from earlier data with mice. Further studies are needed to elucidate the exact metabolic role of Cat K in "OCN - insulin axis" in humans, especially the effect of its pharmaceutical inhibition on pancreatic insulin secretion.

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Overproduction of hepatokine selenoprotein P contributes to hypoadiponectinaemia in type 2 diabetes

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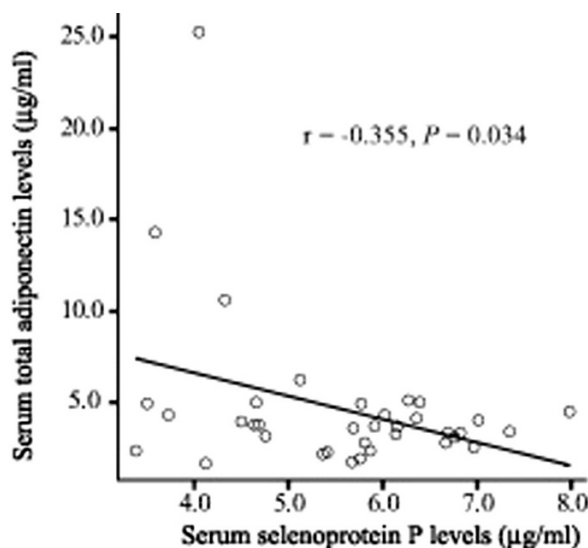
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Background and aims: We have recently identified selenoprotein P (SeP) as a liver-derived secretory protein that causes insulin resistance in the liver and skeletal muscle. We have reported that serum levels of SeP are elevated in patients with type 2 diabetes compared with control subjects. Additionally, we have shown that SeP impairs insulin signal transduction in the hepatocytes and myocytes. However, it is unknown whether and, if so, how SeP acts on adipose tissue. The present study tested the hypothesis that SeP is related to hypoadiponectinemia in patients with type 2 diabetes.

Materials and methods: We compared serum levels of SeP with those of adiponectin and other clinical parameters in 36 patients with type 2 diabetes. Additionally, we treated 3T3-L1 adipocytes with purified SeP to assess the direct action of SeP on adiponectin production. We also measured levels of blood adiponectin in SeP knockout mice.

Results: Circulating SeP levels were positively correlated with fasting plasma glucose ($r = 0.35$, $P = 0.037$) and negatively associated with both total and high-molecular adiponectin in patients with type 2 diabetes ($r = 0.355$, $P = 0.034$; $r = 0.367$, $P = 0.028$). SeP was a predictor of high-molecular adiponectin, independently of age, body weight, and quantitative insulin sensitivity index ($\beta = -0.357$, $P = 0.017$). Treatment with purified SeP protein concentration-dependently reduced adiponectin and PPAR γ gene expression in 3T3-L1 adipocytes. SeP knockout mice exhibited an increase in blood adiponectin levels when fed regular chow or a high sucrose, high fat diet.

Conclusion: These results reveal that overproduction of liver-derived secretory protein SeP contributes to hypo adiponectinemia in patients with type 2 diabetes, and suggests that the failure of the inter-organ network, via hepatokines and adipocytokines, underlies the pathology of type 2 diabetes.



Inverse correlation between serum levels of selenoprotein P and those of adiponectin in patients with type 2 diabetes.

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Increased plasma levels of nesfatin-1 in patients with newly diagnosed type 2 diabetes mellitus

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Background and aims: Nesfatin-1, derived from the nucleobindin2 (NUCB2) precursor, containing 82 amino acid, is highly conserved in humans, rats and mice. It has been reported that nesfatin-1 suppresses nocturnal food intake and body weight gain when injected into the third brain ventricle, conversely, infusion of NUCB2 antisense oligonucleotide stimulates food intake. However, its patho-physiological role in humans remains unknown. The aim of the present study was to investigate plasma nesfatin-1 levels and the association between plasma nesfatin-1 levels and various metabolic parameters in humans.

Materials and methods: 54 subjects with newly diagnosed type 2 diabetes mellitus (nT2DM), 53 subjects with impaired glucose regulation (IGR) and 53 control subjects were enrolled in this study. Plasma nesfatin-1 level was measured by ELISA. The relationship between plasma nesfatin-1 levels and metabolic parameters was also analyzed.

Results: plasma nesfatin-1 levels were elevated in subjects with both nT2DM and IGR compared to the controls (1.91 ± 0.79 vs 1.80 ± 0.80 vs 1.41 ± 0.58 $\mu\text{g/L}$, $P < 0.01$). Simple regression analysis showed that in subjects with IGR and nT2DM, plasma nesfatin-1 positively correlated with body mass index (BMI), Glycosylated hemoglobin (HbA_{1c}), fasting blood glucose (FBG), 2 h blood glucose after glucose overload (2h-PBG), fasting plasma insulin (FINS), the homeostasis model assessment of insulin resistance (HOMA-IR), multivariate logistic regression analysis revealed that plasma nesfatin-1 was significantly associated with IGR and nT2DM, even after controlling for anthropometric variables and lipid profile.

Conclusion: plasma nesfatin-1 concentrations were found to be elevated in subjects with both IGR and nT2DM and to be related to several clinical parameters known to be associated with insulin resistance.

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Vaspin regulates insulin sensitivity

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Background and aims: The novel adipokine vaspin (visceral adipose tissue derived serine protease inhibitor) is suggested to link obesity, insulin resistance (IR) and type 2 diabetes (T2D), but so far its pathophysiological role remains largely unknown. We treated db/db-mice to study the effects of recombinant vaspin on insulin sensitivity. In human studies we analyzed the relationship between serum vaspin concentrations and metabolic traits. Finally, we investigated whether variability in vaspin serum concentrations might be explained by its genetic variants.

Materials and methods: In animal studies, five db/db-mice for each test were treated with vaspin (1mg/kg body weight i.p.) or saline in the control group. Then glucose tolerance tests (2g/kg body weight i.p.) and hyperinsulinemic-euglycemic-clamps were performed. In human genetic studies vaspin was sequenced in DNA samples from 48 unrelated Caucasian subjects. Twenty-eight single nucleotide polymorphisms (SNPs) representative for their linkage disequilibrium groups ($r^2 > 0.8$ and minor allele frequencies > 0.05) were genotyped in 1046 clinically well-characterized Sorbs from Germany for subsequent association studies on metabolic traits including IR and insulin secretion indices. Serum vaspin concentrations were determined by ELISA.

Results: Treatment of db/db-mice with recombinant vaspin resulted in an improved glucose tolerance and an increased glucose infusion rate during the steady state of the clamp (all $P < 0.05$). In human studies, serum vaspin levels correlated with gender, waist-to-hip-ratio (WHR), 2-hr glucose, insulin (fasting, 30min, 2-hr), HOMA-IR and QUICKI (all $P < 0.05$). Additionally there was a strong association of vaspin SNPs with serum vaspin concentrations (with six SNPs reaching P-values between 10^{-8} and 10^{-14}). Also, genetic variants were nominally associated with WHR, 30min glucose, 2-hr insulin, AUC_{Glucose} and IR indices (adj. for age, sex and BMI).

Conclusion: Our data demonstrate the insulin sensitizing effect of vaspin. Moreover, they suggest a role of vaspin genetic variants in the pathophysiology of IR, which might be mediated through their effects on vaspin serum concentrations.

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Pigment epithelium-derived factor is elevated in adipocyte of streptozotocin-induced diabetic Sprague-Dawley rats and inhibited after insulin treatment

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Background and aims: Pigment epithelium-derived factor (PEDF) is recently identified as an adipokine of obese mice to induce insulin resistance (IR), the regulation of its own expression is largely unknown. Our previous studies suggest that early insulin therapy in type 2 diabetes mellitus subjects improve IR and decrease free fatty acids in the circulation. In this study, we aims to investigate the effects and underlying mechanisms of early insulin therapy on PEDF expression in white adipose tissue (WAT) of high fat diet and Streptozotocin (STZ)-induced diabetic Sprague-Dawley rats.

Materials and methods: We examined PEDF expression in fat tissue of STZ-induced diabetic rats before and after Neutral protamine Hagedorn insulin administration and in 3T3-L1 cell models after TNF- α and dexamethasone (DEX) treatments.

Results: Compared with normal ones, PEDF mRNA and protein expressions of WAT of untreated diabetic rats were increased by 9.2 and 4.1 fold, respec-

tively ($P<0.01$). After insulin administration, PEDF decreased significantly. Serum PEDF level was higher in diabetic rats, and inverted after insulin administration. Interestingly, the expressions of TNF- α , IL-6, iK κ B in WAT were up-regulated in untreated diabetic rats. They were down-regulated by the insulin treatment. Treatment of 3T3-L1 cells with TNF- α induce IR at the level of insulin-stimulated glucose uptake ability, which is paralleled by increased PEDF secretion and expression ($P<0.01$). This increase was also attenuated by insulin treatment. Time course study in vitro indicate that insulin decrease PEDF mRNA expression from 15min, 30min, 60min, while has no effects on PEDF expression after treatment for 2h. Using specific inhibitors, we found that PEDF expression was inhibited by insulin through PI3K-Akt signaling pathway, and NF-KB inhibitors PDTC can inhibit PEDF expression induced by TNF- α . PEDF expression was also increased by DEX ($P<0.05$) in fully differentiated 3T3-L1 cells in a time and dose dependent manner, which was attenuated by combination of Dex and insulin together.

Conclusion: PEDF level is elevated in both WAT of diabetic rats and 3T3-L1 cells with IR, which might through inflammatory factor NF- κ B signaling pathway. Insulin therapy might improve the insulin sensitivity by inhibiting PEDF expression.

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Impact of Apo-CIII and Apo-E on insulin sensitivity: data from the RISC study

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Background and aims: Apolipoproteins may affect insulin sensitivity in opposite directions. ApoCIII knock-out mice develop insulin resistance and diet-induced obesity, while ApoE knock-out mice are protected. By inhibiting lipoprotein lipase, Apo-CIII might reduce tissue non-esterified fatty acids (NEFA) availability, whereas ApoE may induce lipid infiltration by promote lipoproteins transfer to extravascular tissues. We undertook to verify whether, and to what extent, Apo-CIII and ApoE modulate the impact of lipids on insulin sensitivity in man.

Materials and methods: In 1,017 non-diabetic subjects from RISC cohort (556 women and 461 men, age 44 ± 8 years, BMI 25.3 ± 3.8 kg/m²), we measured insulin sensitivity (euglycaemic clamp), physical activity (accelerometry), lifestyle habits (questionnaire), blood pressure (Omron), body composition (Tanita), lipid profile and apolipoproteins CIII and E (Multiplex suspension array technology, Luminex).

Results: HDL-cholesterol and triglyceride levels were related to insulin sensitivity, with similar strength but opposite sign ($r=+0.32$ and -0.30 , $p<0.001$ for both). In univariate analysis, ApoE and ApoCIII were only weakly associated with insulin sensitivity ($r=-0.14$ and -0.07 , respectively, $p<0.05$) despite their strong correlation with triglycerides ($r=+0.42$ and $r=+0.54$, $p<0.001$ for both). After adjusting for triglycerides, the association with insulin sensitivity was lost for ApoE (partial $r=-0.01$), while it became positive and stronger for ApoCIII (partial $r=+0.13$, $p<0.001$). The impact (standardised β coefficient) of the triglycerides adjusted ApoCIII on insulin sensitivity ($+9.5\pm 2.3$ $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{f}}^{-1}$, $p<0.0001$) was still positive and statistically significant ($+8.6\pm 2.9$ $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{f}}^{-1}$, $p<0.003$) after further adjusting for centre, age, sex, waist, blood pressure, smoking, physical activity and alcohol consumption. Interestingly, the removal of ApoCIII from the above mentioned model attenuated the negative impact of triglycerides on insulin sensitivity changing their standardized β coefficient from -19.9 ± 3.5 to -13.1 ± 2.2 to $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{f}}^{-1}$ ($p<0.05$). The ratio of serum NEFA to serum triglycerides (an index of intravascular lipolysis) showed a progressive decline through ApoCIII quartiles, however both ApoCIII and triglycerides, although attenuated, remained significant and opposite predictors of insulin sensitivity after adjusting also for serum NEFA.

Conclusion: The negative association of Apo-E with insulin sensitivity appears to be mediated through triglycerides levels. In contrast, Apo-CIII exerts an independent and positive effect on insulin action by attenuating the negative metabolic impact of triglycerides. ApoCIII inhibition of intravascular lipolysis of triglyceride-rich lipoproteins may at least in part explain this association.

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Plasma levels of N ϵ -(carboxymethyl)lysine (CML) are lower in impaired glucose metabolism and type 2 diabetes and this is partly explained by obesity: the Hoorn and CODAM studies

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Background and aims: Increased formation of advanced glycation endproducts (AGEs) constitutes a potential mechanism by which hyperglycaemia and its immediate biochemical sequelae induce micro- and macrovascular complications in diabetes. In type 1 diabetes, circulating levels of AGEs, including the major AGE N ϵ -(carboxymethyl)lysine (CML), have been shown to be increased and associated with the development of cardiovascular risk. In contrast, the exact role of circulating CML in type 2 diabetes (T2DM) remains unclear, because remarkably, so far no consistent data on levels of plasma CML have been described in individuals with vs. without T2DM. We have therefore investigated: 1) the extent to which levels of CML residues in plasma protein differed across different levels of impaired glucose metabolism and 2) the role of T2DM-related risk factors herein.

Materials and methods: We measured levels of CML residues in plasma protein, with the use of HPLC-tandem MS, in 1265 (568 women) individuals from two large population-based cohort studies in the Netherlands; subjects mean age was 64 ± 8 years, 46% of whom had normal glucose metabolism (NGM), 23% had impaired glucose metabolism (IGM) and 31% had T2DM. Data were analysed with multiple linear regression models.

Results: After adjustment for age, sex, prior CVD, HbA1c and use of glucose-lowering treatment, levels of CML were progressively lower in individuals with IGM and T2DM as compared with those with NGM (p -value for trend <0.001 ; Figure-model 1). These differences were significantly attenuated by about 50 and 47%, respectively, when further adjusted for waist circumference (Figure-model 2). Further adjustment for other risk factors (i.e. blood pressure, total:HDL cholesterol ratio, triglycerides, smoking and eGFR) and use of anti-hypertensive or lipid-lowering treatment did not materially change these differences, however (Figure-model 3). Similar results were found when analyses were stratified according to study population.

Conclusion: Levels of CML residues in plasma protein decrease with increasing levels of impaired glucose metabolism and this seems to be partly explained by the increasing levels of central obesity in these individuals. The underlying mechanisms explaining these observations and the role of (central) obesity herein require further investigation.

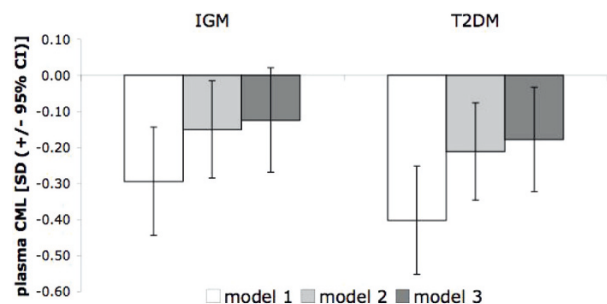


Figure. Differences between IGM or T2DM vs. NGM in plasma CML.

Model 1 is adjusted for age, sex, prior CVD, HbA1c and use of glucose-lowering treatment; model 2 is additionally adjusted for waist circumference; model 3 is the fully adjusted model (i.e. additionally adjusted for blood pressure, total:HDL cholesterol ratio, triglycerides, smoking, eGFR and use of anti-hypertensive or lipid-lowering treatment).

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PS 043 Islet cell function *in vivo*

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Divergent effects of μ -opioid antagonism on insulin secretion from human islets and efficacy in type 2 diabetic patients

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Background and aims: Opioid receptors have been implicated in both weight and glycaemic control in rodents and humans. Recently, μ opioid receptor knock-out mouse data and early clinical data with naloxone suggest that antagonizing opioid receptors may improve islet function and insulin secretion in humans. We have therefore evaluated 1) what are the opioid receptor subtypes expressed in human islets; 2) what is the direct effect of opioid receptor activation and blockade on insulin secretion in isolated human islets; and 3) to what extent opioid receptor blockade acutely improves islet function and glycaemic control in a pilot study in a cohort of type 2 diabetic patients.

Material and methods: Rat islets were prepared from Sprague-Dawley rats and used the day after isolation for insulin secretion. Human islets were procured from cadaver donors that had volunteered for organ donor programs and allowed to recover in culture overnight prior to secretion experiments or RNA extraction. In the human study, which ran in Singapore, patients were evaluated using a blinded randomized cross-over design where post-prandial blood glucose and insulin following a test meal were measured in each patient. All patients received placebo, naloxone, methylnaltrexone or repaglinide in a random order.

Results: In human islets the μ -opioid receptor was the predominantly expressed opioid receptor with no detectable transcripts of other opioid receptors (δ , κ & λ). Human islets furthermore expressed the POMC gene that may be processed to the opioid receptor ligand endorphin. No detectable levels of the PENK gene (enkephalin) were found. The opioid receptor agonist loperamide (1 μ M) suppressed insulin secretion from rat islets by $\approx 75\%$. In human islets 10 μ M loperamide suppressed insulin secretion by $\approx 50\%$. An opioid receptor antagonist was able to reverse the inhibition seen with loperamide. In a cohort of type 2 diabetics, who were randomized in a cross-over study design to placebo, repaglinide, naloxone or the peripherally restricted opioid antagonist methylnaltrexone, we failed to observe any improvements in post-prandial insulin or C-peptide levels over placebo following a test meal with naloxone or methylnaltrexone treatment. In contrast, repaglinide treatment (the positive control) significantly enhanced plasma insulin and C-peptide levels by 72% and 40%, respectively ($P < 0.002$).

Conclusion: Our data suggests that μ opioid receptors are expressed in human islets. As μ -opioid receptors are known to couple through an inhibitory G protein, this is the likely mechanism by which loperamide suppresses insulin secretion *in vitro*. However, in a clinical setting, it appears unlikely that inhibiting the islet opioid receptor pool with an opioid receptor antagonist acutely enhances glycaemic control in type 2 diabetic patients.

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Autocrine inhibitory effects of proinsulin C-peptide and insulin on insulin secretion and pancreatic beta cell function

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Background and aims: C-peptide and insulin are released from pancreatic islets in equimolar amounts after proinsulin is processed by prohormone convertases and carboxypeptidase E. Many regulatory peptides are known to have a feedback mechanism however there is a lack of consistent evidence demonstrating the intra-islet effects of C-peptide and insulin. The present study was undertaken to explore the actions of proinsulin C-peptide and insulin on pancreatic beta cell function.

Materials and methods: Insulin releasing activity of C-peptide (human, rat-1 and rat-2) and insulin were studied in pancreatic islets isolated from normal Swiss TO mice ($n=5$) and results confirmed using the clonal pancreatic cell line, BRIN-BD11. Acute insulin secretion studies were performed at 5.6mM and 16.7mM glucose. A range of C-peptide and insulin concentrations were tested with various insulin secretagogues. Insulin and C-peptide release were both measured by radioimmunoassays. *In vivo* glucose and insulin responses were assessed in fasted Swiss TO mice following i.p. glucose (18mmol/kg) and C-peptide (25nmol/kg) or insulin (25nmol/kg).

Results: Acute exposure of isolated islets to insulin resulted in inhibition of alanine(10mM)-stimulated C-peptide release at 5.6mM glucose (10^{-9} - 10^{-6} M, $p < 0.05$ - $p < 0.001$) and IBMX (200 μ M)-stimulated C-peptide release at 16.7mM glucose (10^{-10} - 10^{-6} M, $p < 0.05$ - $p < 0.001$). Inhibition of C-peptide secretion in the presence of insulin (3×10^{-7} M) was accompanied by suppression of the insulin-secretory response to alanine (10mM, $p < 0.05$) and GLP-1 (10^{-8} M, $p < 0.05$) at 5.6mM glucose, and IBMX (200 μ M, $p < 0.001$) at 16.7mM glucose. Like insulin, in isolated islets, C-peptide (3×10^{-7} M) inhibited insulin secretion at 5.6mM ($p < 0.001$) and 16.7mM ($p < 0.001$) glucose. Inhibition of glucose-induced insulin secretion with C-peptide (3×10^{-7} M) was accompanied by suppression of the insulin-secretory responses to alanine (10mM, $p < 0.01$), GIP (10^{-8} M, $p < 0.01$) and tolbutamide (200 μ M, $p < 0.01$) at 5.6mM glucose. Likewise, C-peptide (3×10^{-7} M) inhibited insulin release induced by IBMX (200 μ M, $p < 0.05$) at 16.7mM glucose. Similar results were found using clonal beta cells and the observations made with human C-peptide were reproduced using rat C-peptide-1/-2. Insulin (3×10^{-7} M) reduced cAMP production in the presence of glucose (16.7mM, $p < 0.001$), GLP-1 ($p < 0.01$) and forskolin ($p < 0.001$) whereas C-peptide (3×10^{-7} M) had no effect. Acute exposure of clonal beta cells to insulin resulted in a decrease in insulin mRNA expression in the presence of alanine at 5.6mM ($p < 0.01$) and 11.1mM ($p < 0.01$) glucose. Human C-peptide (3×10^{-7} M) had no effect. In mice, administration of insulin together with glucose decreased plasma glucose at 15min ($p < 0.001$), 30min ($p < 0.001$) and 60min ($p < 0.001$), compared to glucose alone. Area under the curve (AUC) confirmed reduced plasma glucose ($p < 0.001$) and C-peptide ($p < 0.01$). Similarly, the glycaemic response was impaired by administration of C-peptide (1.3-fold, $p < 0.001$). AUC analysis revealed a decreased insulin response following administration of C-peptide ($p < 0.001$).

Conclusion: This study provides evidence that insulin and C-peptide exert inhibitory effects on pancreatic beta cell function and glucose-stimulated insulin release mediated through intracellular signalling pathways, contributing to the multifactorial regulation of the pancreatic beta cell.

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Insulin-induced stimulation of insulin secretion, its relation to insulin sensitivity and impact on impaired glucose regulation

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Background and aims: In islets of mice with selective knock out of beta-cell insulin receptors, glucose-induced insulin release is impaired. Recent work has shown that insulin stimulates its own secretion in humans and has suggested that insulin resistance in the beta cell could be an important cause of beta-cell dysfunction. We have analysed the RISC study database to test whether insulin exposure and insulin sensitivity modulate beta-cell function in subjects with normal glucose tolerance (NGT) and contribute to the dysglycaemia of subjects with impaired glucose regulation (IGR, impaired fasting glycaemia and/or impaired glucose tolerance).

Materials and methods: Insulin sensitivity (M, by euglycaemic insulin clamp), endogenous insulin release during the clamp (as C-peptide concentration percent change from basal; positive change = stimulation, negative = suppression) and beta-cell glucose sensitivity (β GS, by modelling of the C-peptide response during the OGTT) were measured in 1151 NGT and 163 IGR men and women.

Results: In NGT, insulin-induced suppression of C-peptide was strongly inversely related to both M and the steady-state plasma insulin concentration during the clamp. In a multivariate model controlling for centre, sex, age, BMI and steady-state plasma glucose levels, each standard-deviation increment in plasma insulin and M was associated with a 5% and 23% increase in the C-peptide response, respectively ($p=0.008$ and $p<0.0001$). The C-peptide response was positively, if weakly, related to β GS ($r=0.16$, $p<0.0001$). Both the C-peptide response ($-16 \pm 5\%$ vs $9 \pm 2\%$, $p<0.0001$) and β GS (81 ± 7 vs 139 ± 3 pmol min⁻¹ m⁻² mM⁻¹, $p<0.0001$) were decreased in IGR in comparison with NGT. After adjustment for confounders, M and β GS were the major determinants of mean OGTT glucose levels in both NGT and IGR; however, the contribution of the latter was significantly ($p<0.05$) greater in IGR than NGT (59% vs 38% for each standard-deviation decrement in β GS), with a minor role of the C-peptide response. In a multivariate logistic model, IGR was predicted by β GS (odds ratio = 4.84 [95% CI = 2.89-8.09]) and M (odds ratio =

3.06 [95% CI = 2.19–4.27]) but not by the C-peptide response (odds ratio = 1.11 [95% CI = 0.77–1.61]).

Conclusions: Pre-exposure to physiological hyperinsulinaemia stimulates insulin secretion to a degree that is strongly dependent on insulin sensitivity. However, this phenomenon has limited impact on beta-cell dysfunction and dysglycaemia.

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***In vivo* activation of PKA in beta cells rescues glucose intolerance by enhancement of insulin secretion with no effect on beta cell mass**

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Background and aims: β -cell dysfunction and insulin resistance contribute to impaired glucose tolerance (IGT) and precede the onset of type 2 diabetes mellitus (T2DM). Potential for β -cell based therapies have focused upon increasing insulin secretion pharmacologically or expanding β -cell mass. However, certain diabetic therapies that target the β -cell, such as sulfonylurea treatment, raise the risk of hypoglycemia and lose efficacy over time, while expanding β -cell mass in mice leads to hyperinsulinemia and β -cell tumorigenesis. Here a mouse model (β -caPKA mice) of tamoxifen inducible, β -cell specific constitutive protein kinase A (PKA) activity exhibited improved and sustainable β -cell function through enhanced insulin secretion with unaltered β -cell mass.

Materials and methods: Tamoxifen was i.p. injected into β -caPKA mice and three littermate control lines to activate the constitutively active PKA transgene in β -caPKA mice. IPGTTs were assessed using 1 g/kg body weight in overnight fasted mice and sampling from the tail vein. β -cell mass was determined in histological sections by quantifying insulin positive versus total pancreas area, and total pancreatic insulin content was measured.

Results: Intraperitoneal glucose tolerance tests (IPGTT) after tamoxifen administration revealed an increase in plasma insulin levels in β -caPKA mice versus wildtype controls at 2 (2251 ± 547 pg/ml vs. 658 ± 190 pg/ml; $p < .05$) and 5 (1507 ± 377 pg/ml vs. 635 ± 184 pg/ml; $p < .05$) minutes with no differences observed at later timepoints. This enhancement in glucose-stimulated insulin secretion in β -caPKA mice resulted in a statistically significant decrease in blood glucose levels from 10–60 minutes versus controls, indicating improved glucose tolerance. These effects were sustainable up to 52-weeks of age with no indications of hyperinsulinemia, hypoglycemia, beta-cell exhaustion, or the development of insulinomas. To determine whether enhanced insulin secretion could overcome pre-existing glucose intolerance, β -caPKA mice and wildtype controls were placed on a 45% high fat diet (HFD) for 16 weeks after which tamoxifen was administered. IPGTTs administered 4 weeks later after continual high fat feeding revealed increased insulin secretion at the 2 (4844 ± 1828 pg/ml vs. 951 ± 388 pg/ml; $p < .05$) and 5 (3438 ± 1297 pg/ml vs. 1073 ± 438 pg/ml; $p < .05$) minute timepoints in β -caPKA mice versus wildtype controls with no differences at later timepoints. This enhancement of insulin secretion improved glucose tolerance in 34-week old, high fat fed β -caPKA mice (177 ± 72 mg/dl at 20 minutes) versus wildtype controls (411 ± 168 mg/dl at 20 minutes), and remarkably restored glucose tolerance to the same level observed in 9-week old, chow fed β -caPKA mice (246 ± 82 mg/dl at 20 minutes). β -cell mass increased after high fat feeding but was not different between β -caPKA mice and wildtype controls.

Conclusions: Increasing PKA activity in the β -cell leads to enhanced insulin secretion *in vivo* and was achieved without indications of hyperinsulinemia of hypoglycemia, β -cell exhaustion or the development of insulinomas. Glucose intolerance induced by HFD was fully reversed following tamoxifen induction of the increased β -cell PKA activity in β -caPKA mice, with no differences on β -cell mass between wildtype controls. Thus therapies that solely target β -cell function can fully reverse glucose intolerance without the adverse consequences associated with other treatment strategies.

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Analysis of glucose homeostasis in p66-ko mice reveal a major role of p66shc in nutrient sensing

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Background and aim: The longevity determinant p66shc has a role in insulin signaling and contributes to peripheral insulin desensitization and to the establishment of type 2 diabetes in obese mice. Although the recently discovered biochemical linkage with the mammalian Target of Rapamycin (mTOR) and its downstream nutrient sensitive effector S6 kinase (S6K) suggest the involvement of p66 also in “normal” glucose metabolism and in glucose sensing and insulin secretion by pancreatic beta cells, these possibilities still need to be verified.

Methods and aims: In order to investigate the relevance of p66shc to glucose homeostasis in healthy animals, lean WT and p66KO mice were subduced to a) euglycemic/hyperinsulinemic clamp and b) to hyperglycemic clamp, the two “gold standard” assays for, respectively, peripheral insulin sensitivity and pancreatic beta cell secretory response.

Results: Peripheral response to Insulin was significantly upregulated in p66KO mice compared to wild type controls (glucose uptake: 141.9 ± 62.3 versus 56.1 ± 23.4 mg/Kg⁻¹/min⁻¹, $p < 0.02$), a finding also corroborated by a significant reduction in the level of basal fasting glycemia (85 ± 7.9 mg/dl versus 108.5 ± 9.8 mg/dl) in the presence of comparable plasma insulin, in this strain. Interestingly, under hyperglycemic stress, a clear tendency to a reduced insulinemic response, both in the early (15') and late phase (50–90') of the curve, was observed in p66-deficient mice, consistent with a blunted response of beta cells to glucose load.

Conclusion: If confirmed in larger numbers of animals, this complex phenotype, that strikingly mirrors that of mice harboring defects in the mTOR/S6K signaling cascade, suggests that p66shc, by interfering with nutrient sensing, regulates both tissue response to insulin and glucose-induced insulin secretion. These findings have major implications for the genetics and treatment of type 2 diabetes.

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Deletion of beta-arrestin-2 impaired glucose induced insulin secretion from pancreatic islet cells

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Background and aims: Beta-arrestin-2 is an adaptor protein with multiple functions. Deficiency of beta-arrestin-2 in mice has been reported to induce glucose intolerance by disturbance of insulin signaling. Since beta-arrestin-2 is also expressed in pancreatic beta-cells, the aim of the study was to determine the effect of beta-arrestin-2 on function of pancreatic beta-cells.

Materials and methods: Beta-arrestin-2 knockout (KO) and littermate wild-type (WT) mice were used for the present study. Hyperglycemic clamps were performed over a 90-min period as previously described on 6h-fasted mice to evaluate the beta-cell function *in vivo*. The targeted glucose levels were 16–18 mmol/L. Islets from beta-arrestin-2 KO mice were isolated to measure glucose and forskolin (FK) induced insulin release.

Results: Beta-arrestin-2 protein expression increased in pancreatic islet cells from high fat diet mice. As previous reported glucose intolerance was observed in beta-arrestin-2 deficient mice compared with WT although the 6h-fasting insulin level was comparable between KO and WT mice. Hyperglycemic clamp test showed that both the acute phase ($AUC_{0-20min}$) and late phase insulin secretion ($AUC_{20-90min}$) decreased from KO mice (acute phase: 4.17 ± 0.63 ug/L; late phase: 11.08 ± 1.71 ug/L) compared with those from WT mice (acute phase: 11.69 ± 2.80 ug/L; late phase: 29.61 ± 5.31 ug/L) (all $p < 0.01$, $n = 7-12$). No difference could be found in insulin content from pancreas between KO and WT mice. To understand the role of beta-arrestin-2 on beta cell function, islets from beta-arrestin-2 KO mice were isolated and cultured at low (G6.1) and high glucose (G20) concentrations overnight. Consistent with the finding *in vivo*, glucose induced insulin release decreased ($p < 0.05$, $n = 6$) in beta-arrestin-2 KO mice (53.73 ± 10.08 ug/mg protein) compared with wild type littermates (28.34 ± 6.66 ug/mg protein). Moreover, insulin secretion induced by FK was blunted from KO islets (42.37 ± 9.01 ug/mg protein) compared with that from WT islets (75.61 ± 15.62 ug/mg protein) ($p < 0.02$, $n = 5$).

Conclusion: The results indicated that beta-arrestin-2 was involved in insulin secretion stimulated by glucose.

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Toxic consequences of the atypical maturation and disposal process of proinsulin in the *Ins2^{+/-Akita}* beta cells

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Background and aims: Proinsulin is the most abundant insulin precursor made in β -cells. The processing of proinsulin molecules involves a complex process of “folding.” Until recently, this was felt to occur very rapidly, resulting in formation of mature insulin molecules. We recently reported that proinsulin preserves an aggregation-prone nature and a low relative folding rate that result in production of abundant non-natively folded non-monomer (i.e., aggregate) forms. Thus, proinsulin maintains a homeostatic balance of natively and non-natively folded states (i.e., proinsulin homeostasis, PIHO) in normal β -cells as a result of the integration of disposal and maturation processes. We have also revealed the susceptibility of PIHO to various (non)genetic factors. Based on our accumulated evidence, we have suggested that PIHO, an early post-translational regulation mechanism in insulin biosynthesis, may critically link to diabetes. To provide supporting evidence for this hypothesis, in this study we characterized what cytotoxic consequences result from the primarily disturbed PIHO in the *Ins2^{+/-Akita}* β -cells with a genetic disorder (C96Y) in the *Ins2* gene.

Materials and methods: We applied quantitative PCR, metabolic-labeling, immunoblotting, and other biochemical and morphological studies in the cloned *Ins2^{+/-Akita}* and control *Ins2^{+/+}* β -cell lines. Statistical analyses are performed using Student's t-test (2-tailed) or analysis of variance if appropriate, with $P < 0.05$ considered statistically significant.

Results: We have found that the primarily disturbed PIHO in the *Ins2^{+/-Akita}* β -cells results in a decrease ($P < 0.01$) in the levels of cellular (pro)insulin and secreted insulin, and increases ($P < 0.01$) of cellular (pro)insulin disposals and the secreted proinsulin/insulin ratio notwithstanding no obvious attenuations of transcription and translation. There are other consequences in the *Ins2^{+/-Akita}* β -cells. These include the enlargement of endoplasmic reticulum (ER), Golgi, and mitochondria organelles, the decrease of insulin granules in the number and size, and the increase ($P < 0.01$) of the reactive oxygen species, thioredoxin-interacting protein, tyrosine-nitrated proteins, and β -cell death rate.

Conclusion: Our studies demonstrate that the abnormalities in insulin production and mitochondrial morphology, ER and/or oxidative stress, and β -cell depletion resulted directly from PIHO disorders without secondary contributions of glucotoxicity. This is because that the cloned *Ins2^{+/-Akita}* and control *Ins2^{+/+}* β -cells were subjected to same concentrations of glucose in culture and to same treatments in this study. The data suggest that proinsulin maturation and disposal disorders (induced by various factors) results in a number of consequences that contribute to the development of β -cell failure and diabetes.

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Delta-cell secretory responses to insulin secretagogues are not mediated indirectly by insulin

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Background and aims: Little is known about the regulation of somatostatin (SST) secretion from pancreatic delta cells although a range of insulin secretagogues has been reported to stimulate SST release. The aim of the study was to assess whether the similarity in the secretory response from delta and beta cells is due to an indirect effect of insulin on SST release.

Materials and methods: Mouse islets were isolated by collagenase digestion. Hormone secretion from primary islets or the TGP52 delta-cell line was assessed in static incubation studies. Insulin, glucagon and SST release was measured by radioimmunoassay (RIA).

Results: Incubation with exogenous insulin (100 nmol/l) did not modulate glucose-induced SST release from mouse islets (1 mmol/l glucose (G):

1.14±0.04 fmol SST/islet/h; 1 mmol/l G + insulin: 1.02±0.08; 20 mmol/l G: 2.98±0.27; 20 mM G + insulin: 2.75±0.36, $P > 0.2$ vs. 1 and 20 mmol/l G, respectively). The lack of effect was not due to binding of endogenous insulin to the insulin receptor since preincubation with either the insulin receptor tyrosine kinase inhibitor HNMPA(AM)₃ (10 μ mol/l, 1 h) or an insulin receptor antibody (IRAB, 1 μ g/ml, 48h) did not alter the secretory response of the islets to glucose (1 mmol/l: 0.97±0.08 fmol SST/islet/h; 1 mmol/l + HNMPA(AM)₃: 1.27±0.25; 1 mmol/l + IRAB: 1.21±0.16; 20 mmol/l: 1.95±0.22; 20 mmol/l + HNMPA(AM)₃: 1.64±0.32; 20 mmol/l + IRAB: 2.47±0.22; $P > 0.2$). In the same experiments HNMPA(AM)₃ did not modulate glucose-induced inhibition of glucagon secretion (1 mmol/l G: 23.9±1.8 pg/islet/h; 1 mmol/l G + HNMPA(AM)₃: 21.2±2.4; 20 mmol/l G: 13.4±1.4; 20 mmol/l G + HNMPA(AM)₃: 12.8±1.4; $P > 0.2$ vs. controls) nor did IRAB alter the response to glucose or arginine (20 mmol/l G: 48.7±3.5; 20 mmol/l G + IRAB: 37.2±3.2; 20 mmol/l arginine: 122.8±11.5; 20 mmol/l arginine + IRAB: 113±7.8, $P > 0.08$ vs. 20 mmol/l G, $P > 0.2$ vs. 20 mmol/l arginine). In contrast, glucose-induced insulin secretion was significantly enhanced following preincubation with HNMPA(AM)₃ (20 mM G: 2.53±0.27; 20 mM G + HNMPA(AM)₃: 4.28±0.34; $P < 0.001$) although no effect was observed with IRAB (20 mmol/l G: 2.08±0.27; 20 mmol/l G + IRAB: 1.79±0.35; $P > 0.2$). To exclude other potential interactions between beta- and delta-cells, secretion experiments were carried out with the TGP52 delta cell line to assess whether the SST secretory profile was maintained in the absence of any beta cell input. Similar to the response of primary islets, glucose and tolbutamide stimulated SST release from TGP52 cells (1 mmol/l G: 3.9±0.16 fmol SST/100,000 cells/h; 20 mmol/l G: 7.45±0.49; 100 μ mol/l tolbutamide: 8.85±0.87, $P < 0.001$) and glucose-induced secretion was inhibited by 10 μ mol/l NA (20 mmol/l G + NA: 5.35±0.13 fmol/100,000 cells/h; $P < 0.01$).

Conclusion: These data imply that the similarity in secretory responses of beta- and delta-cells is not due to an indirect action of insulin (or other beta-cell specific secretory products) on the delta-cell. Alternatively, similar stimulus-response coupling pathways are operating in the two different islet cell types. Our results also indicate that endogenous insulin is not mediating the inhibitory action of glucose on glucagon secretion but may negatively regulate insulin release.

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In situ electrophysiological examination of pancreatic α cells in type 1 diabetes revealing the cellular basis of glucagon hypersecretion

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Aims: To characterize voltage-gated ion channels in α cells of Type 1 Diabetic (T1D) mouse model and understand how these channels are altered to collectively lead to hyperglucagonemia in the early stage of diabetes.

Materials and methods: GYY mouse model, which expresses EYFP specifically in pancreatic α cells, was employed to facilitate the identification and localization of α cells. T1D was induced by low dose (40mg/kg) Streptozotocin (STZ) treatment for 5 consecutive days. T1D development was monitored by fed and fasting blood glucose, blood glucagon levels and IPGTT. Islet α cell mass and morphology by confocal microscopy and total pancreatic glucagon content were assessed. Pancreas tissue slices were prepared from control and diabetic mice to enable in situ examination of a cell electrophysiology by standard whole-cell patch-clamp technique.

Results: STZ-treatment caused T1D phenotype including fed and fasting hyperglucagonemia (control vs. STZ-group; fasting: 67±3.4 vs. 121±20 pg/ml; fed: 128±11.6 vs. 220±32.7 pg/ml, $P < 0.05$), hyperglycemia (control vs. STZ-group; fasting: 11.7±1.7 vs. 17.7±1.7 mmol/L; fed: 10.1±0.5 vs. 28.7±1.5 mmol/L, $P < 0.001$) and glucose intolerance measured by IPGTT. While 71% of β cell mass was ablated by STZ treatment, a cell mass was not significantly changed in diabetic mice (control vs. STZ-group; 2.9±0.5 vs. 2.4±0.24 mg). However, total pancreatic glucagon content was elevated in T1D (control vs. STZ-group; Total-Gluc: 4.6±0.3 vs. 27.6±9.1 pg/ μ g, $P < 0.001$). Taken together with the normal α cell size in the diabetic mice as measured by membrane capacitance (C_m) (control vs. STZ-group; 4.7±0.2 vs. 4.9±0.2 pF), this indicates that each α cell contained elevated glucagon content. Single α cell glucagon granule exocytosis examined by serial depolarization-induced ΔC_m was increased in α cells of diabetic mice. Consistently, static incubation of pancreas

slices at stimulatory 3mM glucose showed higher glucagon secretion from the diabetic α cell populations. Electrophysiological studies on individual α cells suggested that α cells in diabetic mice expressed larger voltage-gated Na current (control vs. STZ-group; 72.2 ± 7.6 vs. 86.7 ± 10.0 pA/pF, $P < 0.05$). Voltage-gated K current was significantly reduced in α cells of diabetic mice (control vs. STZ-group; 485 ± 46 vs. 416 ± 35 pA/pF, $P < 0.05$). Membrane potential recording revealed increased action potential (AP) duration, amplitude and firing frequency in α cells of diabetic mice.

Conclusions: Hyperglucagonemia in T1D is contributed by the following perturbation in α cell physiology. First, each α cell in T1D has upregulated glucagon content that can be released when triggered. Second, α cell membrane ion channel machinery (AP firing frequency, amplitude and duration) is primed for glucagon release in response to low glucose stimulation. Third, the primed glucagon release is contributed by the larger Na current that sensitizes towards action potential firing, and reduced voltage-gated K current which slows membrane repolarization after AP firing thus prolonging AP duration.

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Systemic effects of liver regeneration on rat beta cells and islets: *in vivo* and *in vitro* studies

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Background and aims: Liver regeneration induces the release of humoral factors *in situ* and in blood circulation possibly influencing pancreatic function. Liver regeneration involves adaptation of glucose metabolism. This work studies the systemic effect of liver regeneration on the native pancreas in normal rats. *In vitro*, the outcome of rat RINm5F beta cells, grown in standard medium supplemented with normal or diabetic hepatectomized (Hx) rat serum and insulin secretion of Hx rat islets are examined.

Materials and methods: 2/3 partial hepatectomy was performed in normal and streptozotocin induced diabetic Lewis rats (n=6). Pancreas from control, SHAM or Hx normal rats were weighted 1, 2 or 3 days after surgery, islets size examined by insulin staining. RINm5F cells were grown in RPMI medium containing 10% serum from Hx or SHAM rats. After 24 h, cell viability and count were evaluated by CellTiter^R and cell counter. Glucose-stimulated insulin release from islets isolated three days after Hx or control rats was studied. **Results:** The ratio pancreas/body weight was higher in Hx rats compared to SHAM, 3 days after surgery: 0.58 vs 0.39 (p=0.057). After partial hepatectomy, proportion of small size islets (<10 000 μ m²) increased significantly from 76% to 90% (p < 0.05) (control vs 3 days post Hx). By comparison with the serum obtained from Sham animals, Hx normal rat serum promoted a significant increase in RINm5F cell viability: 126% vs 94% (p=0.005) and cell proliferation : 4.62 vs 3.54×10^5 cells / ml (p=0.036) (n=6), with serum withdrawn 2 days after surgery. Conversely, Hx diabetic rat serum withdrawn 3 days after hepatectomy had an opposite effect with significant decrease in cell viability: 79 vs 95% (p=0.036). Insulin release in low-glucose medium trends to be higher for Hx rats islets compare to control rats islets: 28 vs 16 ng/ml/10 islets p=NS. No difference was found for insulin stimulation index.

Conclusion: Liver regeneration induces systemic effects on pancreatic islets and increases their viability in normal rat but not in diabetic rat. Further studies on islet functionality must be achieved on rat islets isolated after partial hepatectomy.

PS 044 Hypoglycaemia: clinical aspects

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A higher counter-regulatory hormone response is seen with insulin degludec than insulin glargine in response to induced hypoglycaemia in type 1 diabetes

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Background and aims: Intensified insulin therapy can often have side effects, of which hypoglycaemia remains the most prominent. An appropriate hormonal counter-regulatory response plays an important role in glucose recovery. We conducted a double-blind, two-period, crossover trial to investigate the counter-regulatory hormone responses elicited during a period of hypoglycaemia induced by insulin glargine (IGlar) or the ultra-long-acting basal insulin, insulin degludec (IDeg).

Materials and methods: A total of 28 subjects with type 1 diabetes (age 41 ± 12 years, diabetes duration 21 ± 10 years, HbA_{1c} $7.8 \pm 0.6\%$) were randomised to receive either IDeg or IGlar once daily for 5 days. At midnight on Day 5, each individual received an insulin dose which was three times higher than their personal daily requirement. A variable intravenous glucose infusion was used to maintain euglycaemia at 5.5 mmol/l for the next 7 hours. After this period, glucose infusion was terminated to lower plasma glucose (PG) to 3.5 mmol/l (kept for a duration of 30 min) and then to 2.5 mmol/l (for 15 min). PG was then raised to 3.9 mmol/l, where it was maintained for 2 h, before a further elevation to 5.5 mmol/l. Hormonal counter-regulation was assessed at baseline and at PG levels from 4.5–2.5 mmol/l.

Results: Rate of PG decline and the lowest PG level obtained were comparable between IDeg and IGlar. Noradrenaline and glucagon responses were similar for both IDeg and IGlar. However, levels of both growth hormone and cortisol secretion during hypoglycaemia (from PG 4 mmol/l to 2.5 mmol/l) were significantly higher with IDeg compared with IGlar, with respective AUC_{hormone} [ng*min/ml] values of 498 vs. 204 (treatment ratio (TR) IDeg/IGlar: 2.44 [1.30; 4.60] p<0.01) for growth hormone and 10834 vs. 8808 (TR: 1.23 [1.01; 1.50] p<0.05) for cortisol. In addition, there was an observed trend towards a higher adrenaline response for IDeg compared with IGlar, which failed to reach statistical significance (AUC [pg*min/ml]: 6636 vs. 4726 (TR: 1.40 [0.96; 2.04] p=0.07)). Under hypoglycaemic conditions (PG 2.5 mmol/l), glucose infusion rates for both insulins were comparable; however, less glucose was required to alleviate hypoglycaemia with IDeg than with IGlar during the recovery phase (AUC_{GIR} [mg/kg]: 201 vs. 285, respectively, (TR: 0.71 [0.53; 0.93] p<0.02)).

Conclusion: In people with type 1 diabetes, induction of hypoglycaemia with IDeg results in a significantly greater counter-regulatory hormone response compared with IGlar. This observation indicates that enhanced counter-regulation may contribute to the observed reduced hypoglycaemia risk for IDeg compared with IGlar.

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Prolonged hypoglycaemic activity of the novel insulin analogues: preliminary study on animal diabetes model

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Background and aims: Using modified insulin with a potentially prolonged pharmacological action, that could maintain basal level of glucose for more than one day, seems to be interested and advantageous in clinical practice. The objective of the study was to evaluate pharmacological effects of the new long-acting insulin analogues, taking into account particularly the length of the action after stopping their administration.

Materials and methods: New recombinant, human insulin analogues, coded GKR, GEKR and AKR of the potential long-acting characteristics were evalu-

ated in our institute. In each of the analogue two aminoacid residues were added to C-terminus of B chain (B31Lys- B32Arg). Additional changes referred to B3 and A22 positions. These modifications have resulted in shifting of the isoelectric point to the highest values (6.6–7.0) comparing to human insulin. The study examined single-dose and chronic (21 or 28-day) hypoglycemic action of the new analogues on the hyperglycemic animal model (streptozotocin rats) in comparison to the control group of physiological salt solution (0.9% sodium chloratum). Experimental hyperglycemia was induced by streptozotocin in doses ranging from 32 to 40 mg/kg b.w (mimicked mild to severe form of clinical diabetes). The overall glycemic profiles up to 36 h were evaluated after a single subcutaneous dose of the study preparations. In the multiple-dose experiment preparations were administered 2 or 3 times daily to maintain anticipated glucose levels close to normoglycaemia. Glycemia in steady-state was controlled once-a-day in the morning before the first daily dose of an analogue. Observation was continued and one-daily glycaemia measurement was performed up to 11 days (in case of GKR) and up to 14 days (in case of GEKR and AKR) after stopping the analogues administration. Some safety aspects have also been assessed. Histology observations of skin, subcutaneous tissue and main internal organs were carried out after the termination of pharmacological experiments.

Results: The multiple-dose experiment confirmed with statistical significance hypoglycaemic properties of GEKR, GKR and AKR insulin in comparison to the control group (Newman-Keuls test; $p < 0.05$ in case of each analogue). After stopping each analogue's injection the prolonged hypoglycemic effect was stable and observed up to 11 or 14 days. The concentrations of blood glucose in subsequent days were statistically different than glucose level in the control group ($p < 0.05$ in each case). Means values of glucose concentrations in the subsequent days ranged 126–146, 148–198 and 165–222 mg/dl in case of GKR, GEKR and AKR, respectively. No macroscopic and microscopic pathological changes in the injection site and in all tested organs were found.

Conclusion: The prolonged activity of insulin analogues is supposed to be due to formation of a very stable deposit in the site of administration. Because of the isoelectric point of about 7, solubility of the new insulin analogues decreases in physiological pH and this probably causes their precipitation at the subcutaneous tissue and slow release to the blood thanks to which a therapeutic level may be much longer maintain. The tested insulin are candidates for even less frequent subcutaneous administration than once-a-day.

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Duration of diabetes and hypoglycaemia rates in type 2 diabetes patients treated with insulin glargine vs NPH insulin

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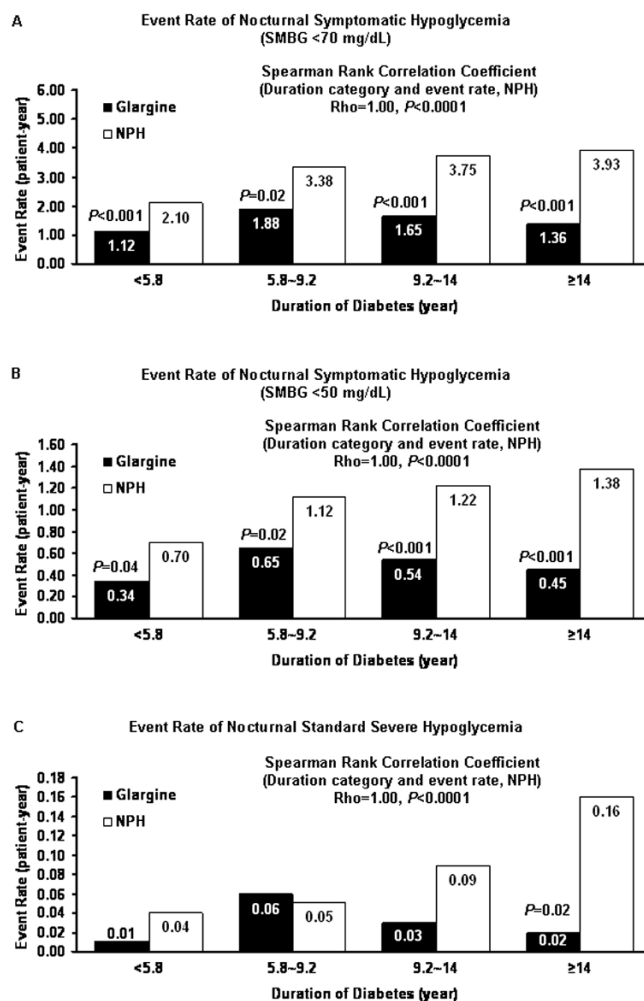
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Background and aims: Recent ACCORD results suggest the incidence and risk of hypoglycemia increases with duration of type 2 diabetes (T2D). This analysis examined whether there were differences in hypoglycemia rates as a result of T2D duration in patients treated with glargine (GLAR) vs NPH.

Materials and methods: Data were pooled from four 24-wk randomized controlled trials of insulin-naïve T2D patients comparing GLAR + oral antidiabetic drugs (OADs) vs qd NPH+OADs. Patients were stratified into quartiles by T2D duration: <5.8 years (GLAR: n=378; NPH: n=285); 5.8–9.2 years (n=330 and 269, respectively); 9.2–14 years (n=289, 276); and ≥14 years (n=261, 242).

Results: Of 2330 patients, 1258 received GLAR, 1072 NPH. Baseline demographics were similar except T2D duration in the highest quartile (GLAR: 19.64 years, NPH: 18.25 years; $P=0.002$). Despite similar or better HbA_{1c} levels at endpoint for each quartile, GLAR patients had fewer episodes of nocturnal hypoglycemia (SMBG <70 mg/dl) vs NPH ($P < 0.05$ for all). With duration increased, patients on GLAR had less nocturnal hypoglycemia at successively lower blood glucose (BG) cut-offs, eg, patients with T2D for ≥14 years had significantly fewer severe nocturnal hypoglycemic episodes defined as SMBG <50 mg/dl or <36 mg/dl despite a better endpoint HbA_{1c} for patients with ≥14-years T2D duration (7.71% GLAR vs 7.93% NPH; $P=0.031$) with similar insulin dose (mean insulin dose GLAR vs NPH: 33 vs 34 U). Nocturnal hypoglycemia increased significantly only with NPH with increasing T2D duration (for NPH: SMBG <70 mg/dl, <50 mg/dl, and severe hypoglycemia; Rho (Spearman Correlation Coefficient)=1, $P < 0.0001$ [Figure]).

Conclusion: We conclude that with longer T2D duration, patients taking GLAR had significantly less nocturnal hypoglycemia compared with NPH at successively lower BG thresholds.



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Clinical predictors of risk of hypoglycaemia during addition and titration of insulin glargine for type 2 diabetes

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Background and aims: Adding and titrating basal insulin can restore HbA_{1c} to target levels for many patients with inadequate control of type 2 diabetes (T2D) with oral agents alone. A leading barrier is hypoglycemia which halts titration and may put patients at risk. Identifying patients with high risk of hypoglycemia might assist in titration and early addition of prandial insulin.

Materials and methods: Seeking clinical predictors of hypoglycemia, we analyzed data for 2251 patients (45% women, mean age 58 years, duration of DM 8.9 years, BMI 31.0 kg/m², taking 0–2 oral agents) in 11 studies with at least 24-week use of insulin glargine in a treat-to-target dosing scheme. Hypoglycemia was systematically identified and classified as symptomatic, confirmed by glucose <50 mg/dL, or severe (3rd party assistance).

Results: The % of patients affected (incidence) was 51.6, 25.4, and 1.5 for symptomatic, confirmed, and severe hypoglycemia, respectively. Mean event-rates/participant-year were 6.46, 1.30, and 0.06, respectively. Values were entered into multivariate statistical models. Model 1 included age, gender, duration of diabetes and oral therapy, BMI, baseline HbA_{1c} and FPG, and use of a sulfonylurea with metformin vs metformin alone. Model 2 added endpoint HbA_{1c}. Incidence of symptomatic hypoglycemia was independently associated with age, duration of diabetes, lower BMI, and use of a sulfonylurea with

metformin vs metformin alone. HbA_{1c} after insulin glargine treatment was also independently associated, with 1/3 increased risk per 1% lower HbA_{1c} . After adjustment for attained HbA_{1c} associations of baseline, predictors with hypoglycemia were equal or increased.

Conclusion: 1) Lower HbA_{1c} after treatment, consistent with intensive titration of dose, increased the risk of hypoglycemia. 2) Independent of attained HbA_{1c} , younger age, longer duration of diabetes, less adiposity, and use of a sulfonylurea with metformin predicted higher risk.

Incidence of Symptomatic Hypoglycemia

Independently associated covariates	Model 1 Baseline Factors		Model 2 Model 1 + Endpoint HbA_{1c}	
	Odds ratio	p-value	Odds ratio	p-value
Age (yr)	0.989	0.0337	0.987	0.0107
Duration of diabetes (yr)	1.015	0.0610	1.021	0.0089
BMI (kg/m^2)	0.962	<0.0001	0.963	0.0001
Metformin vs. metformin + sulfonylurea	0.238	<0.0001	0.209	<0.0001
Endpoint HbA_{1c}	-	-	0.669	<0.0001

Supported by: sanofi-aventis, US

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Are older patients with type 2 diabetes and insulin therapy under worse glycaemic control?

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Background and aims: Poor metabolic control and increased frequency of hypoglycaemia are considered to arise from diminishing competence in personal management and cumulative multimorbidity in elderly patients with insulin therapy. In well-known clinical studies for the treatment of type 2 diabetes mellitus (T2DM) the influence of patients' age is not specifically examined: UKPDS <65y, Kumamoto <70y, ACCORD 62y, ADVANCE 66y, VDAT 60y, 4T 62y. For evaluating these assumptions we investigated the incidence of any hypoglycaemia and clinical characteristics of insulin treated patients with T2DM.

Materials and methods: By assessing the frequency of any hypoglycaemia for the preceding 12 months in 393 insulin treated patients with T2DM aged 41 to 90 years at a university outpatient department we used a standardised questionnaire and interrelated with clinical and laboratory data drawn from the electronic patient record EMIL. Patients were separated on the basis of the median age, 68 years. Non-severe hypoglycaemia was defined as either a condition with symptoms in conjunction with hypoglycaemia and rapid attenuation after carbohydrate ingestion or a plasma glucose test below 2.2 mmol/l without the necessity of showing symptoms. Severe hypoglycaemia was defined as the imperative for an i.v. injection of glucose or a dose of glucagon intramuscular. Each patient had a structured education in the last 20 years. HbA_{1c} was DCCT adjusted.

Results: The patients had a significantly longer period since diagnosis (<68y: 15±7y; ≥68y: 20±9y; $p<0.01$) and a meaningful lower BMI (<68y: 34±6 kg/m^2 ; ≥68y: 32±5 kg/m^2 ; $p<0.01$). Neither did HbA_{1c} differ (<68y: 6.8%; ≥68y: 6.7%), nor the frequency of non severe hypoglycaemia (<68y: 0.29/pat/week; ≥68y: 0.22/pat/week) or of severe hypoglycaemia in the last 12 months (0.03/pat/y; both). The blood glucose threshold for symptoms of hypoglycaemia was also similar (<68y: 3.8 mmol/l; ≥68y: 3.6 mmol/l). Taking non severe hypoglycaemia as dependent variable and adjusting for insulin dose, gender, HbA_{1c} and age, the linear regression analyses revealed significant associations for more frequent severe hypoglycaemia in the last 12 months ($p<0.01$), a longer time since diagnosis ($p<0.01$), a major fear of hypoglycaemia ($p<0.01$) and a lower BMI ($p=0.046$). In linear regression analyses with HbA_{1c} as dependent variable increasing insulin dose ($p<0.01$), a lower incidence of severe hypoglycaemia during the last 12 months ($p<0.01$) and female gender ($p=0.045$) are significantly associated after adjustment time since diagnosis, fear of hypoglycaemia, BMI, non severe hypoglycaemia and age.

Conclusions: Despite extended duration of disease and in contrast to clinical experience, hypoglycaemia did not occur more often in elderly patients with insulin treated T2DM. Furthermore HbA_{1c} has not increased, even though previous studies proved its rising in age even in sound persons. The applica-

bility of these findings for general practitioners' patients or retirement home residents needs to be additionally evaluated.

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Association between less physical activity level and reports of hypoglycaemia in patients with type 1 diabetes mellitus

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Background and aims: Daily practice of physical activity (PA) and exercise are recommended for individual with diabetes mellitus (DM), however, this could predict hypoglycemia. The present study aim to evaluate cross-sectionally the association between PA level and report of hypoglycemia episodes in patients with type 1 DM.

Materials and methods: Outpatients with type 1 DM followed consecutively in Endocrinology Division had PA level assessed by the International PA Questionnaire (long form) and the patients were classified in less, moderate, and the very active. The patients answered questions about self-care related to exercise practice and moderate and/or severe hypoglycemia episodes in the last six months. The Ethical Committee of Hospital de Clínicas approved this protocol.

Results: One hundred and twenty six patients with type 1 DM [35 (28-47) years old; 55% women; HbA_{1c} 9.3 ± 2.1%; BMI 25.0 ± 4.2 kg/m^2] were classified according to PA in less active [$n = 15$ (12%)], moderate active [$n = 81$ (64%)], and very active [$n = 30$ (24%)]. The PA realized at work-related and transport-related PAs were the principal factors to determine the PA level of moderate and very active patients ($P<0.05$; Kuskall-Wallis test). Very active patients presented lower values of plasma glucose (150 ± 80 mg/dL vs. 216 ± 113 mg/dL; $P=0.023$) and LDL-cholesterol (178 ± 49 mg/dL vs. 187 ± 46 mg/dL; $P=0.011$) as compared to less active patients [ANOVA test (LSD post-hoc)]. A smaller proportion of the very active patients (33%) reported moderate and/or severe hypoglycemic episodes in the last six months when compared with less active patients (60%) and moderate active (58%; X^2 test; $P=0.056$). Bigger proportion of moderate active patients (48%) and very active patients (67%) practiced an exercise regularly as compared to the less active patients (13%; X^2 test; $P=0.003$). Considering only the 61 patients who have practiced exercise regularly: 22 patients have received specific advises about self-care and 28 patients realized some care before, during, and after exercise, without difference between the groups (X^2 test). Among the self-care, the food intake was the most cited (25%), followed by diet and capillary glucose monitoring (21%). Regression model was constructed to evaluate the possible association between PA level and report of hypoglycemia episodes (dependent variable). Less active patients presented three times more chance of reported episodes of hypoglycemia when compared with very active patients (OR 3.49; IC 95% 1.26-9.70; $P=0.016$), adjusted for fasting glucose, diet, and exercise practice. Alpha <0.05 (18.0 SPSS version).

Conclusion: Most patients with type 1 DM were classified as active, however due to the work-related and transport-related PAs and not by regularly exercise practice. Few patients have taken self-care about hypoglycemia prevention related with exercise practice. Outpatients with type 1 DM and less active presented more chances of hypoglycemia report, probably by the practice of informal moderate/vigorous PA without specific self-care.

Clinical Trial Registration Number: 08612

Supported by: FIPE - Hospital de Clínicas de Porto Alegre

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The relationship between HbA_{1c} values and the occurrence of hypo- and hyperglycaemia as assessed by continuous glucose monitoring in patients with type 1 diabetes

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Background and aims: To examine the relationship between the occurrence of hypo-/hyperglycaemia and HbA_{1c} values, as assessed by continuous glucose monitoring (CGM) in patients with type 1 diabetes receiving basal-bolus insulin therapy (excluding CSII).

Materials and methods: The study's subjects comprised a total of 71 patients with type 1 diabetes receiving basal-bolus insulin therapy, who were put on a CGMS GOLD (Medtronic, Inc., Northridge, CA) immediately after admission. The subjects were divided into four groups by HbA_{1c} (NGSP value) at

admission: group A, those with HbA1c up to 7%; group B, those with HbA1c > 7% and up to 8%; group C, those with HbA1c > 8% and up to 9%; and group D, those with HbA1c > 9%. The first serial measured values over a 24-hour period in these patients were subjected to analysis, with hypoglycaemia and hyperglycaemia defined as HbA1c 180 mg/dL, respectively, to examine how HbA1c values might be associated with the frequency of hypoglycaemia episodes. Additionally, all groups were compared for 24-hour mean glucose levels and their standard deviations (SDs), duration of hypoglycaemia, duration of nocturnal hypoglycaemia (from 23:00PM to 06:00AM) and duration of hyperglycaemia.

Results: The study's subjects comprised 15 patients in group A, 22 patients in group B; 15 patients in group C; and 19 patients in group D. The median (25–75th percentile) values for the subjects in groups A to D were as follows: age, 37 (33–59), 40 (29–58), 35 (30–43) and 40 (32–58); BMI (kg/m²), 19.6 (17.7–21.5), 21.1 (19.2–23.1), 22.7 (21.2–24.7) and 21.3 (20.2–23.6); urinary CPR (μg/day), 1.4 (0.8–7.2), 1.1 (0.5–6.4), 1.1 (0.6–2.7) and 1.3 (0.6–10.3). Of the variables, only BMI was significantly different between the groups ($P = 0.012$; Kruskal–Wallis test). CGM data obtained over a 24-hour period in groups A to D were as follows: 24-hour mean glucose level (mg/dL), 134 (111–156), 153 (122–178), 168 (149–200) and 180 (138–208); SD of the mean glucose level, 61 (40–70), 55 (47–65), 63 (46–67) and 59 (46–80); duration of hypoglycaemia (min), 175 (80–350), 100 (0–260), 50 (0–105) and 70 (0–255); duration of nocturnal hypoglycaemia (min), 130 (45–280), 35 (0–155), 0 (0–106) and 0 (0–110); and duration of hyperglycaemia (min), 355 (110–595), 400 (213–624), 540 (395–860) and 520 (350–910), respectively. A comparison between the groups showed that significantly shorter hypoglycaemia duration was seen in group C than in group A ($P = 0.015$; Mann–Whitney test); and that hyperglycaemia occupied significantly longer in group C than in groups A and B ($P = 0.042$, $P = 0.038$, respectively; Mann–Whitney test). Bimodal logistic regression analyses with independent variables defined as age, BMI, urinary CPR, HbA1c values and SDs demonstrated that the presence or absence of hypoglycaemic episodes and nocturnal hypoglycaemic episodes alone were significantly correlated with HbA1c values, with the odds ratio for hypoglycaemia being 0.584 ($P = 0.008$) and the odds ratio for nocturnal hypoglycaemia being 0.646 ($P = 0.021$).

Conclusion: Of the type 1 diabetic patients stratified by HbA1c values, duration of hypoglycaemia in those with HbA1c > 8% and up to 9% were significantly shorter than those with HbA1c up to 7%. It was also shown that an increase in HbA1c values of 1% was associated with a 42% reduction in the relative risk for hypoglycaemia and a 35% reduction in the relative risk for nocturnal hypoglycaemia.

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Factors associated with hypoglycaemic blood glucose levels two hours after a 75g oral glucose load: Does stature matter? The DEPLAN study
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Background and aims: The observation of low plasma glucose levels, often within the hypoglycemic range, is a common phenomenon during an oral glucose tolerance test (OGTT). Although the upper limits of normal for OGTT are well-standardized, the significance of low glucose values obtained during testing and their relation to body size is less clear. Short height has been related to increased incidence of impaired glucose tolerance, especially in women. Aim of the present study was to investigate possible anthropometric and clinical factors associated with the occurrence of low blood glucose levels (≤ 70 mg/dL) after an OGTT.

Materials and methods: The study participants were generally healthy individuals who underwent an OGTT during a screening procedure for participation in a type 2 diabetes prevention program by lifestyle modification (the DEPLAN study). Hypoglycemia was defined as a plasma glucose value ≤ 70 mg/dL (3.89mmol/L). Only individuals with normal glucose tolerance status were included in the present analysis. A subgroup of the participants underwent a second OGTT, after three years of follow-up.

Results: Out of 577 individuals (242 [41.94%] males, aged 54.37 [53.52–55.21] years) included in the present analysis, 108 (18.71%) had a hypoglycemic 2-hour post-load glucose value. These individuals, compared to those without 2-hour hypoglycemia (and normal glucose tolerance), were males in a higher proportion (66 [61.11%], OR: 2.62 [1.70–4.02], $P < 0.001$), they were younger (52.41 [50.80–54.01] vs. 54.81 [53.84–55.79] years, $P = 0.01$) had lower BMI (27.75 [27.00–28.49] vs. 29.07 [28.62–29.52] Kg/m², $P = 0.03$), lower

fasting plasma glucose (91.43 [89.82–93.03] vs. 94.61 [93.85–95.35] mg/dL, $P < 0.001$) and higher stature (1.72 [1.70–1.73] vs. 1.66 [1.65–1.67], $P < 0.001$). There was no difference between the two groups in terms of blood pressure, waist circumference, physical activity and family history of diabetes. In multivariable analysis (logistic regression), higher stature (OR: 1.08 per 1cm increase [1.05–1.11], $P < 0.001$) and lower fasting plasma glucose (OR: 1.48 per 10mg decrease [0.95–2.06], $P = 0.001$) were independent predictors of 2-hour post-load hypoglycemia occurrence. Gender differences disappeared after the introduction of height in the model.

Conclusion: High stature predicts independently hypoglycemic plasma glucose levels 2 hours after an OGTT. The reason is not clear, although it may be hypothesized that taller persons have more muscle tissue for uptake of a fixed (75g) amount of glucose.

PS 045 Risks and costs of hypoglycaemia

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Incidence and costs of severe hypoglycaemia in diabetes requiring attendance by the emergency service in the United Kingdom

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Background and aims: Symptomatic hypoglycaemia is an important adverse effect of therapy for diabetes. We determined the incidence of severe hypoglycaemia in a defined population, its management, and the costs associated with requests for emergency assistance from the ambulance service.

Materials and methods: Over a twelve-month period, December 2009 to November 2010, sequential, routinely collected datasets were analysed to measure the incidence of severe hypoglycaemia, defined as requiring emergency assistance from the South Central Ambulance Service NHS Trust. The Trust serves a population of four million people in the south of England, United Kingdom (UK) within which there are estimated to be 161,000 people diagnosed with diabetes over the age of 17 years. Numbers of episodes, management and use of hospital services were identified for calls to those aged >1 year. Costs of accident and emergency department attendance and primary care follow up were estimated using Personal Social Services Research Unit costs 2010. Costs of emergency call, attendance at the scene and conveyance to hospital were provided by the ambulance trust

Results: During the survey period, 398,409 emergency calls were received, of which 4081 (1.02%) were recorded with hypoglycaemia as the 'chief complaint'. 53.8% (2108) of calls related to men, with the age distribution of patients and the number of emergency calls for hypoglycaemia by age group (years) shown in the table. The incidence of hypoglycaemia was 39/100,000 population for ages 1 to 39 years, and 163/100,000 for those aged 40 years and above. At least one blood glucose measurement was recorded in 83.4% of calls and, where recorded, the initial median (IQR) glucose level was 2.3 (1.7) mmol/l. 65.7% of those attended were given carbohydrates, oral or intravenous glucose, or glucagon. 1441 (35.3%) of those attended were taken to hospital. The estimated total cost (£'000s) of initial ambulance attendance and treatment at scene was £909; if transport to hospital was necessary, the additional ambulance transport costs were £223 plus emergency department costs of £140; and the cost of primary care follow-up was estimated as a further £0.62. The average cost per emergency call was £326.

Conclusion: This is the largest UK survey to describe the incidence of severe hypoglycaemia requiring an emergency ambulance attendance, with an estimated annual cost of £16.9 million for England. The highest numbers of incidents involving hypoglycaemia are in older age groups. Despite overall improvements in glycaemic control, there remains a substantial burden from hypoglycaemia.

Emergency calls for hypoglycaemia by age group (years)

Age Group	Number of calls for patients in age group	Percentage of all calls
1 to 19	207	5.1
20-39	591	14.5
40-59	1083	26.6
60-79	1330	32.7
≥80	862	21.2

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Comparison of hypoglycaemia risk and cost between oral antidiabetic monotherapies in elderly patients with type 2 diabetes mellitus in a U.S. population

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Background and aims: The objectives of the study were to assess the risks and annual healthcare costs of hypoglycemia associated with oral antidiabetic drug (OAD) monotherapy types in the elderly.

Materials and methods: We conducted a retrospective analysis of the IH-CIS/Ingenix impact claims database from January 1999 to September 2008. Patients aged ≥65 years with at least two claims for T2DM diagnosis who received monotherapy with an OAD (sulfonylurea [SU], metformin [MET], or thiazolidinedione [TZD]) were followed for 1 year from the OAD initiation date. DPP4 and meglitinide are not included in the analysis due to small sample sizes. Hypoglycemia was identified using ICD-9 codes of 250.8X, 251.0X, 251.1X, or 251.2X. Rates of hypoglycemia were estimated for each OAD class. Risk of hypoglycemia associated with each OAD in reference to SU monotherapy was examined using a Cox proportional hazard regression model adjusting for patient characteristics and comorbidities. Healthcare costs in the 12 months following the initiation of the OAD were compared descriptively between patients with at least one hypoglycemia event and patients without hypoglycemia using Wilcoxon tests.

Results: The sample included 7,620 SU patients, 6,675 MET patients, and 1,940 TZD patients. The rates of hypoglycemia associated with SU, MET, and TZD at 1 year were 2.7%, 1.5%, and 1.3%, respectively. Both MET and TZD carried a lower risk of hypoglycemia than SU (hazard ratios of 0.63 and 0.49, respectively, $p < 0.001$). Total and diabetes-related healthcare costs were higher in patients with than without hypoglycemia for each OAD (except for total costs in the TZD group; Table).

Conclusion: Among OAD monotherapies, SU poses the highest risk of hypoglycemic events in elderly T2DM patients. Hypoglycemia in all OAD classes is associated with increased healthcare costs.

Descriptive Annual Healthcare Costs

Annual Healthcare Costs (US Dollars, mean±SD)	SU			MET			TZD		
	Hypo-glycemia (N=208)	No Hypo-glycemia (N=7,412)	P	Hypo-glycemia (N=102)	No Hypo-glycemia (N=6,573)	P	Hypo-glycemia (N=26)	No Hypo-glycemia (N=1,914)	P
Total	27,100±36,500	11,460±22,102	<.001	23,620±46,090	9,880±15,656	<.001	18,169±20,268	13,641±26,233	0.183
Diabetes-related	13,834±25,796	3,665±11,710	<.001	11,958±42,141	2,985±8,926	<.001	7,345±11,604	4,216±13,645	0.008

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The impact of glucose variability on achievement of glycaemic control and risk of hypoglycaemia in patients with type 2 diabetes

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Background and aims: Glucose variability has been proposed as a predictor of clinical outcomes in patients with type 2 diabetes (T2D). The impact of glucose variability on glycemic control and hypoglycemia during treatment intensification is unknown.

Materials and methods: We pooled data from 6 randomized controlled clinical trials of insulin glargine (GLAR) vs a comparator (oral agents/other insulins) to determine changes in glucose variability during a treat-to-target (FPG ≤100mg/dL) protocol and to explore the relationships among baseline glucose variability, age, A_{1c}, and hypoglycemia. Glucose variability was calculated from 7-point glucose profiles using standard deviation (SD) and mean amplitude of glycemic excursions (MAGE).

Results: Complete data were available at baseline and 24 weeks for 1699 patients (1026 GLAR, 673 comparator); 43% female, 95% white, mean age 59(9) years and duration of T2D 9(6) years. Mean A1C was reduced from

8.7(.95)% to 7.0(.91)% and FPG from 194(48)mg/dL to 125(38)mg/dL. Glucose variability was significantly reduced on trial in the entire cohort irrespective of treatment type (Table); younger patients (<65 years) experienced twice the reduction of older patients. Patients who failed to achieve A1C $\leq 7.0\%$ had modestly higher baseline glucose variability than those who reached goal. Patients who experienced ≥ 1 symptomatic hypoglycemic event during the study had modestly higher baseline glucose variability than those with no hypoglycemia. Comparable associations of glucose variability, A1C, and hypoglycemia were observed when GLAR patients were analyzed separately.

Conclusion: Our post-hoc analysis shows a significant decrease in glucose variability during therapy with a variety of antihyperglycemic agents, including insulin glargine, and suggests that T2D patients with elevated glucose variability at baseline are at increased risk for hypoglycemic events and greater likelihood of failing to reach A1C $\leq 7.0\%$. Improved understanding of these relationships may lead to better treatment strategies for T2D.

Glucose Variability			
All patients (N=1699)	Change from baseline, mean (SD)		P value
SD	-4.21 (20.2)		<0.001
MAGE	-7.44 (33.2)		<0.001
Relationship of Baseline GV & A1C at 24 Weeks			
	Baseline GV, mean (SD)		LS means* for difference (P value)
All patients (N=1699)	A1C ≤7.0% (N=949)	A1C >7.0% (n=750)	
SD	42.4 (16.8)	44.3 (18.6)	-2.74 (P<0.001)
MAGE	61.7 (27.0)	64.9 (31.1)	-4.57 (P<0.001)
Relationship of Baseline GV & On-Trial Hypoglycemia			
	Baseline GV, mean (SD)		LS means* for difference (P value)
All patients (N=1699)	≥1 hypo event (n=1035)	No hypo (n=663)	
SD	45.8 (18.2)	39.2 (15.8)	4.36 (P<0.001)
MAGE	66.6 (30.5)	57.6 (25.4)	5.38 (P<0.001)
*means adjusted for study			

* means adjusted for study

Supported by: sanofi-aventis, US

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Hypoglycaemia in adult vs. elderly type 2 diabetes mellitus patients: risks, costs, and impact on treatment persistence in a U.S. population

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Background and aims: We compared risks of hypoglycemia associated with various antidiabetic drugs, impact on treatment persistence, and estimated healthcare costs between adult and elderly patients with type 2 diabetes mellitus (T2DM).

Materials and methods: A retrospective analysis of the IHCIS/Ingenix impact claims database from 1/1999 to 9/2008 was conducted in T2DM patients aged ≥ 18 yr with ≥ 1 prescription drug claim for an oral antidiabetic (OAD) and a subgroup of elderly patients aged ≥ 65 yr. Hypoglycemia was identified by ICD-9 codes (ICD-9: 250.8X, 251.0X, 251.1X, or 251.2X). Relative hypoglycemia risks of various drug classes were determined using a treatment- and covariate-adjusted Cox proportional hazard regression model with time-varying covariates measured in 6-month intervals. The impact of hypoglycemia on treatment discontinuation (≥ 30 day gap) was evaluated using adjusted generalized estimating equations. Annual healthcare costs following OAD initiation were compared between patients with and without hypoglycemia using adjusted generalized linear models.

Results: Of 207,748 T2DM patients identified, 30,335 were elderly (≥ 65 years). The hazard ratios for hypoglycemia risk associated with sulfonylureas, metformin and thiazolidinediones were 1.95 ($p<0.0001$), 1.08 ($p=0.0843$), and 1.22 ($p<0.0001$), respectively, in the elderly, and 1.56, 1.18, and 1.09 (all $p<0.0001$), respectively, in all adults, including the elderly. The odds of treatment discontinuation for those with vs. without hypoglycemia in a given 6-month interval were greater in elderly (OR=1.35, $p<0.0001$) than in all adults (OR=1.26, $p<0.0001$). The differences in both all-cause and diabetes-related annual healthcare costs between patients with and without hypoglycemia were greater in elderly (all cause: 20,264 US Dollars [USD] vs. USD 11,897; diabetes-related: USD 11,829 vs. USD 4,190, $p<0.0001$) than adult patients (USD 14,031 vs. USD 9,007 and USD 7,012 vs. USD 3,265, respectively, $p<0.0001$).

Conclusion: Compared to adults, elderly T2DM patients exhibit higher risks of treatment-associated hypoglycemia in most treatment groups. Risk of treatment discontinuation and healthcare costs attributable to hypoglycemia are also higher in the elderly.

Supported by: Takeda Pharmaceuticals International, Inc.

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Hypoglycaemia as a risk factor for mortality

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Background and aims: Hypoglycaemia (blood glucose <4mmol/l) has been shown to be associated with increased mortality and adverse outcomes in patients admitted with pneumonia, critically ill ICU patients and acute myocardial infarction. The aim was to identify whether hypoglycaemia was a risk factor for mortality in patients admitted to the medical assessment unit (MAU) with medical emergencies.

Materials and methods: Retrospective analysis of all patients who attended the MAU and had blood tests from July 2008 till December 2008 through the laboratory data-base. Patients who had documented hypoglycaemia (n=94; 25 with diabetes) were identified. Information gathered through reviewing individual notes to look at the required parameters. We selected 91 age and sex matched controls (n=11 with diabetes) with blood glucose between 4-7mmol/l.

Results: Patients with hypoglycaemia had lower glucose levels [3.4(95% confidence interval(CI):3.3,3.5) vs 5.8(5.7,5.9)mmol/l]. Median follow-up was 1.14 years during which 54 deaths (44.6% in those with hypoglycaemia vs.13.2%, $X^2=24.8$, $P<0.0001$) occurred. Crude mortality rates were 62.6 deaths per 100 person-years in those with hypoglycaemia at admission, compared with 11.7 deaths per 100 person-years in controls. Cases had higher admission levels of creatinine and urea [171(141, 201) vs.100(70,130) and 11.9(10.1,13.8 vs. 6.9(5.1, 8.8)mmol/l] respectively. Hypoglycaemia [Hazard ratio (HR) = 4.58(2.4,8.7), $P<0.0001$], elevated urea [HR:1.07 (1.05,1.09) per 1mmol increase], creatinine [(HR:1.02,(1.01,1.03) per 10mmol/l], potassium [HR:1.83(1.35,2.49)] and sodium levels [HR: 1.05(1.00, 1.10)] were associated with higher rates of mortality in univariate Cox regression analyses. In multivariate models, age, hypoglycaemia (HR: 4.86(95%CI:2.39,9.86) and urea (HR:1.05(1.02,1.08), independently predicted mortality in acutely ill patients.

Conclusion: In-hospital hypoglycaemia is an important risk factor for mortality and these patients need intensive monitoring and treatment. Larger studies are required to confirm these results.

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Underestimated impact of non-severe nocturnal hypoglycaemic events (NHEs) on patients' functioning and well being

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Background and aims: Although non-severe nocturnal hypoglycaemic events, or NHEs, occur fairly frequently in both children and adults, there is a relative lack of literature on the impact of these events on the lives of patients. We used three sources of data in an attempt to assess the impact of non-severe NHEs.

Materials and methods: Our data sources included a systematic literature review using multiple databases of English articles from 1995-2010; qualitative data from 70 patients in focus groups in three countries; and a four-country survey, which was quantitatively analysed for subjects who had experienced a non-severe NHE in the past month.

Results: We identified 32 relevant articles from the literature search (out of a total of 746) which suggested NHEs impact patients' sleep quality, leading to increased fatigue levels and decreased cognitive ability the following day. This was shown to result in increased absenteeism from work and missed appointments. The focus groups revealed that, irrespective of country, NHEs resulted in reduced sleep quality and next-day functioning, and increased concern over their health, particularly with regards to the status of their diabetes. Data obtained from the survey revealed that 70% of the 2,600 respondents reported experiencing a non-severe NHE during the previous month. 54% of these 1,844 patients were in the workforce. 22.7% of these reported that they were either late or missed a full day of work the following day, resulting in an average of 14.7 hours lost due to absenteeism. 31.8% reported that they missed a meeting or work appointment or did not finish a work task on time. Across all

the data obtained, an average of 20% of those who experienced a non-severe NHE contacted a healthcare professional. Of particular concern was the finding that 20% reduced their insulin dose over subsequent days as a result of the event, with clear implications for the management of their diabetes condition. Non-severe NHEs therefore result in lost work productivity and absenteeism, and a change in patient adherence to treatment regimens.

Conclusion: It is clear that the impact of non-severe NHEs is of some significance and it is therefore of some concern that it has been underestimated and underrepresented in the literature. The results of this study demonstrate that greater focus should now fall on this phenomenon, both clinically and in research, in order to ensure that the lives of those affected can be improved.

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Frequency of severe and non severe hypoglycaemia and difficulties at workplace in patients with insulin treated diabetes mellitus

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Background and aims: Hypoglycaemia can lead to impairment and thus could become a threat for the patient himself and for others and can result in difficulties at workplace. Starting insulin therapy may have considerable consequences for drivers or policemen. Therefore some patients with type 2 diabetes refuse insulin therapy and accept hyperglycaemic state with all the risks that go along with it. Current occupational medicine guidelines rely on high prevalence data of hypoglycaemia with 10% of insulin treated patients experiencing at least one severe hypoglycaemia per year and 2-3 events of non severe hypoglycaemia per week. We examined the incidence of severe and non-severe hypoglycaemia in employed patients with diabetes mellitus type 1 (DM1) and 2 (DM2) in Germany to verify if these data are still applicable.

Materials and methods: We assessed any hypoglycaemia in 166 insulin treated patients who are still under employment using a standardised questionnaire at a university outpatient department: 105 patients with DM1 (age 42±11years; diabetes duration 17±12years; BMI 26±5kg/m²; HbA1c 7.0±0.8%), 61 patients with DM2 (age 54±7years; time since diagnosis 12±8years; BMI 33±5kg/m²; HbA1c 6.8±1.0%). All patients had at least one structured education in the last 20 years. Non-severe hypoglycaemia was defined as a condition with symptoms consistent with hypoglycaemia and rapid attenuation after carbohydrate ingestion or a plasma glucose test below 2.2mmol/l even without any symptoms. Severe hypoglycaemia was defined as the necessity for injection of glucose i.v. or glucagon intramuscular. We used a Likert scale from 1 to 6 to judge difficulties at workplace (1: no difficulties; 6: great difficulties). The patients were grouped to no difficulties at workplace (score 1-3) and difficulties at workplace (score 4-6). Clinical and laboratory data are drawn from the electronic patient record EMIL (<http://www.itc-ms.de>). HbA1c was DCCT adjusted.

Results: At least one non-severe hypoglycaemia quarterly occurred in 93% of DM1 and in 43% of DM2. The median frequency per week for all patients was 1.15 (max. 10) in DM1; 0.06 (max. 2.5) in DM2 ($p<0.01$) and the mean frequency per week for those who had at least quarterly hypoglycaemia was 1.89 in DM1; 0.56 in DM2; ($p<0.01$), respectively. Severe hypoglycaemia during the last 12 months occurred in 8% DM1 (0.1/pat/y) but no in DM2 ($p<0.05$). Severe hypoglycaemia occurred in 32% DM1 and 3% DM2 since time of diagnosis. Patients state having difficulties at workplace equally frequent, regardless of DM1 or DM2 (15%; 19%; $p<0.58$). In the ordinal regression analyses only frequency of non severe hypoglycaemia was significantly associated with difficulties at workplace ($p=0.02$). With each non severe hypoglycaemia per week difficulties-at-work-score increased by 0.2 after adjustment for severe hypoglycaemia in the last 12 months, HbA1c, age and diabetes duration.

Conclusion: Frequency of hypoglycaemia in employed patients with diabetes is lower than given in current occupational medicine guidelines. Especially for type 2 diabetes the occurrence of hypoglycaemia is overestimated. Furthermore difficulties at workplace seem more to be the result of an increased frequency of non severe hypoglycaemia and are not so much dependent on severe ones.

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Absence of caspase-1 protects against diet-induced obesity and insulin resistance: identification of underlying mechanisms

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Background and aims: The world-wide epidemic of obesity has accelerated the number of individuals diagnosed with type 2 diabetes. Obesity is characterized by an enlargement of adipose tissue mass that promotes the development of a chronic low grade inflammation. Obesity-induced inflammation leads to enhanced production of numerous pro-inflammatory mediators including IL-18 and IL-1 β that may cause insulin resistance. In order to become active, both IL-18 and IL-1 β are processed by the cysteine protease caspase-1. Previously, we have shown that caspase-1 is activated in adipose tissue of obese animals. Therefore, the aim of this study was to test whether caspase-1 contributes to the development of obesity and insulin resistance and to identify possible underlying mechanisms.

Materials and methods: Both wild-type (Wt) and Caspase-1 $^{-/-}$ animals were fed a high fat diet to induce obesity. Metabolic cage studies, hyperinsulinemic-euglycemic clamps and mechanistic studies on triglyceride (TG) metabolism were performed.

Results: Caspase-1 $^{-/-}$ animals were protected against the development of high fat diet induced obesity. Despite a similar caloric intake (Wt: 11.99 \pm 0.42, Caspase-1 $^{-/-}$: 12.22 \pm 0.53 (kcal/day), ns), HFD-feeding of wild-type mice led to higher plasma insulin (Wt: 6894 \pm 760, Casp-1 $^{-/-}$: 3848 \pm 575 (pg/ml), p -value < 0.001), leptin (Wt: 79684 \pm 11246, Casp-1 $^{-/-}$: 33703 \pm 5629 (pg/ml), p -value < 0.001) and resistin levels (Wt: 5425 \pm 295, Casp-1 $^{-/-}$: 3305 \pm 338 (pg/ml), p -value < 0.01) as compared to Caspase-1 $^{-/-}$ animals. Importantly, Caspase-1 $^{-/-}$ animals were resistant to the development of insulin resistance. Protection against obesity was not caused by a decrease in intestinal TG uptake in Caspase-1 $^{-/-}$ animals, as assessed by gavage of glycerol tri[³H] oleate-labeled olive oil after Triton WR1339 injection. However, oil red O staining of the intestine revealed numerous lipid droplets within the enterocytes of Caspase-1 $^{-/-}$ animals compared to wild-type mice suggestive of abnormal lipid processing. In addition, VLDL-TG production was decreased by -49% ($P<0.01$) in the absence of Caspase-1 together with an impairment in VLDL-TG clearance. Finally, metabolic cage studies revealed an enhancement in total energy expenditure in Caspase-1 $^{-/-}$ animals.

Conclusion: Absence of Caspase-1 protects against the development of diet-induced obesity that may partly be mediated through effects on energy expenditure and a reduced flux of triglycerides from the intestine and liver towards adipose tissue. Inhibition of Caspase-1 may be a useful therapeutic strategy for treatment of obesity.

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Role of MAPK38 and MKK3/6 in the diabetes development

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Background and aims: The obesity and diabetes are associated with increased intracellular p38 mitogen-activated protein kinase (MAPK) signaling, which may promote tissue inflammation and injury. The family members of p38 MAPK are proteins that regulate different and important process like proliferation and apoptosis. These kinases are activated for a double phosphorylation on serine and threonine for MKK3 and MKK6. There are four p38MAPK isoform with different roles in the signaling, p38 α , β , γ and δ . While p38 α and β are the most well known, p38 γ and p38 δ still less understood. In

fact, the p38 α has been implicated in the development of insulin resistance and p38 δ in the production and release of insulin and in the development of obesity-induced pancreatitis. On the other hand, MKK3, the upstream kinase was described that has an important role of declining renal function in diabetic mice, and the other upstream kinase, MKK6 inhibited IRS-1 and IRS-2 expression in adipocytes. The objective of our study is to investigate the role of these kinases in the control of obesity-induced insulin resistance using genetic modified mice

Materials and methods: Mice lacking p38 δ/γ or MKK3-/- MKK6-/+ and WT control mice were fed with high fat diet (HFD) or chow diet for 16 weeks in order to produce obesity-induced diabetes. Body weight, glucose, insulin, and insulin resistance in serum and tissues were analyzed.

Results: After 16 weeks fed with HFD, MKK3-/- MKK6-/+ and p38 δ/γ mice were leaner than WT mice. Analysis of glucose levels in the blood also indicates that MKK3-/-MKK6-/+ and p38 δ/γ develop less glucose intolerance after the HFD.

Conclusion: Lack of the expression of MKK3 and MKK6 results in leaner mice than WT mice after HFD. This could be due to a reduction on the activation of p38 δ/γ by these kinases. In fact, the mice lacking p38 δ/γ have the same reduction on body weight after the diet, indicating that MKK3 and MKK6 exert their action in controlling obesity, at least part, through the activation of p38 δ/γ

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Periportal μ -opioid receptors regulate a neural gut-brain cross-talk between intestinal gluconeogenesis and hypothalamus, controlling energy and glucose homeostasis

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Background and aims: Intestinal gluconeogenesis is a recently described function of control of energy and glucose homeostasis. Glucose produced by the intestine is detected by a portal glucose sensor and activates hypothalamic targets, the signal being transmitted by neural afferents. This may account for the decreased food intake and increased insulin sensitivity observed in protein-fed rats. A number of peptides issued from the partial digestion of proteins have μ -opioid activity (agonists increase food intake and antagonists decrease it). Moreover, μ -opioid receptors (MOR) are widely expressed in the gut. We investigated if MOR modulators could induce their effect via a modulation of intestinal gluconeogenesis.

Materials and methods: Portal infusions of μ -opioid receptor agonists or antagonists were performed for 8 hours in conscious rats fed on normal chow or high-protein diet. Glc6Pase and PEPCK proteins (regulatory enzymes of gluconeogenesis) were quantified as an estimate of gut gluconeogenesis. Comparable experiments were performed with both wild type and MOR-knockout mice. The presence of MOR within portal vein walls (by immunofluorescence) and the impact of infusions on the dorsal vagal complex and hypothalamus (by c-Fos immunohistochemistry) were studied.

Results: On chow diet, Glc6Pase activity was lowered in rats infused with a MOR agonist (4.0 \pm 1.4 U/g of protein for DAMGO vs 9.7 \pm 0.9 U/g of protein for control saline infusion; $P < 0.05$), whereas it was increased in rats infused with a MOR antagonist (17.7 \pm 0.3 U/g for Naloxone, $P < 0.05$ vs saline). These results were correlated with Glc6Pase and PEPCK protein amounts. High-protein diet induced Glc6Pase activity in the intestine vs chow diet (25.4 \pm 2.9 U/g of protein vs chow diet with saline infusion; $P < 0.05$). No differences were found upon antagonist infusion on Glc6Pase activity in high-protein fed rats (24.1 \pm 2.1 U/g of protein for Naloxone infusion, NS vs saline). However, the inhibition by MOR agonist was still occurring (13.1 \pm 1.4 U/g of protein; $P < 0.05$ vs saline infusion). These results were correlated with PEPCK expression. The effects produced by either MOR agonists or antagonists were abolished after portal denervation by capsaicin, suggesting that a central relay involving portal afferents was required to the control of intestinal gluconeogenesis. Immunohistochemistry showed that MOR were tightly co-localized with neurofilaments flanking the portal vein. The number of c-Fos positive cells was 2 to 3 times higher in the Nucleus of the Solitary Tract, the Area Postrema and hypothalamic nuclei of rats infused with MOR-antagonists. The c-Fos increase was abolished everywhere after portal denervation. Proteolytic digests or selected di- or tri-peptides increased Glc6Pase and PEPCK, as did MOR antagonists, in either rats or wild type mice. None of these effects was observed in MOR-KO mice. Finally, MOR-KO mice were

insensitive to the satiety and insulin-sensitization induced by a protein-enriched diet, such as occurring in WT mice.

Conclusion: 1) MOR in the periportal area may initiate a neural circuit of control of intestinal gluconeogenesis having an impact on energy and glucose homeostasis, in addition to their well-known central action on food intake via the reward system; 2) the satiety and insulin-sensitizing effects of protein-enriched diet may be dependent on an antagonistic action on intestinal MOR via their proteolytic digests.

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MIF deficient mice have improved insulin sensitivity and adipose tissue inflammation following high-fat diet

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Background and aims: Adipose tissue inflammation is positively correlated with obesity, insulin resistance (IR) and Type 2 Diabetes. Macrophage Migration inhibitory factor (MIF) is a pro-inflammatory mediator that promotes cytokine secretion and amplifies transmigration, recruitment and activation of leukocytes and is elevated during obesity. Little however is known about the contribution of MIF in development of high-fat diet (HFD) induced obesity and IR. In this study we hypothesize that mice lacking MIF protein will be protected from the adverse effects of high fat diet (HFD) on insulin sensitivity.

Materials and methods: Wildtype(WT) and MIF^{-/-} mice were placed on a HFD (45% palm oil) for 16 weeks. Body weight and food intake was monitored weekly. Insulin sensitivity was assessed at baseline and after high-fat feeding by performing insulin (0.75U/kg insulin) and glucose (1.5g/kg glucose) tolerance tests. Stromal vascular fraction (SVF) isolated from epididymal adipose tissue was stained with antibodies against F4/80, CD11b, and CD11c to identify the number and type of macrophage present and analysed by flow cytometry. SVF cells triple positive (F4/80⁺/CD11b⁺/CD11c⁺) were classified as M1-proinflammatory macrophages and cells double positive (F4/80⁺/CD11b⁺/CD11c⁻) were classified as M2-antiinflammatory macrophages. Cytokine secretion from adipose tissue explants (50mg/1ml media) cultured for 24hours was quantified by Mesoscale multiplex ELISA.

Results: Despite similar food intake MIF^{-/-} mice gain significantly less weight (7g weight difference) than WT mice when fed HFD. MIF^{-/-} mice displayed greater insulin sensitivity and glucose tolerance compared with WT after HFD. M1/M2 macrophage infiltration into adipose tissue was significantly lower in MIF^{-/-} mice compared to WT mice (M1:17.12 \pm 5%;29.93 \pm 5%) (M2:0.861 \pm 0.038%;1.47 \pm 0.038%). Cytokine secretion from adipose tissue explants increased with HFD in WT, but not in MIF^{-/-} mice. The most prominent difference observed was on IL-6 secretion. Weight matched animals displayed identical results.

Conclusion: Observed weight differences suggest MIF plays a key role in energy metabolism. We controlled for weight to identify the specific contribution of MIF on IR and adipose tissue inflammation. Our study supported our hypothesis and demonstrated that lack of MIF protects from diet-induced IR by attenuating macrophage infiltration and reducing adipose tissue inflammation. MIF may serve as a potential biomarker for disease progression or therapeutic target for the treatment of obesity induced IR and Type 2 Diabetes.

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Analysis of TBC1D1 in glucose- and fatty acid-metabolism in an obese mouse model

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Background and aims: TBC1D1 is a Rab-GTPase-activating protein (GAP) with high homology to the insulin signalling protein AS160 (TBC1D4). The highest expression of TBC1D1 can be found in skeletal muscle, lower levels are also expressed in heart, pancreas and hypothalamus. In a positional cloning approach we previously identified a SJL mouse strain-specific loss-of-

function mutation in the *Tbc1d1*-gene that acts as an obesity suppressor. Our data indicated that mutation of *Tbc1d1* in mice was associated with adiposity due to elevated lipid use as well as decreased glucose uptake rates in skeletal muscle. In humans however, a rare *TBC1D1* variant (R125W) could be linked to an extreme form of familial obesity. The exact molecular mechanisms by which *TBC1D1* controls skeletal muscle metabolism and thereby influences whole body energy homeostasis are still unknown. In order to investigate the role of *TBC1D1* in energy homeostasis, we analyzed the impact of *TBC1D1* deletion in an established obese mouse model.

Materials and methods: The mutated *Tbc1d1*-allele from the lean SJL-strain was introgressed into an obese B6.V-Lep^{ob}/J background, a monogenic mouse model for obesity. Body weight and body composition (NMR) were analysed for 15 weeks. The respiratory quotient (RQ) and energy expenditure were measured by indirect calorimetry, food intake and voluntary physical activity (IRmot) was also determined. Carbohydrate- and fatty acid- metabolism were analyzed by measuring 2-desoxy-[1,2-³H]glucose uptake and [1-¹⁴C] palmitate oxidation in intact isolated skeletal muscle (EDL, Soleus).

Results: *TBC1D1*-deficient B6.SJL-Nob1.10-Lep^{ob}-mice had a significantly decreased body weight (49.5±0.6 vs. 45.7±0.8 g; *p*=0.00059) at week 15 which mainly resulted from reduced fat mass. Furthermore *TBC1D1*-deficiency led to a decreased RQ (dark phase: 1.022±0.009 vs. 0.994±0.011; *p*=0.065) which indicates a higher fat utilisation. These mice also displayed increased energy expenditure in the dark phase (2.63±0.03 vs. 2.79±0.03 kJ/g lean mass; *p*=0.0093). Isolated skeletal muscle of *TBC1D1*-deficient mice showed significantly reduced insulin-stimulated glucose uptake in glycolytic EDL muscle (4.63±0.40 vs. 3.26±0.19 nmol/mg/20 min; *p*=0.0019) while basal palmitate oxidation in oxidative soleus muscle was increased (2.09±0.16 vs. 3.11±0.31 pmol/mg/min; *p*=0.0093). There were no differences in food intake at 16 weeks of age (0.116±0.004 vs. 0.112±0.007 g/g body weight) and voluntary physical activity (132187±23875 vs. 158226±41128 beam breaks/24h) in *TBC1D1*-deficient mice compared to the controls.

Conclusion: Our data strongly indicate that *TBC1D1* influences whole body energy homeostasis by regulating the substrate flux of glucose and fatty acids in skeletal muscle. Since a role of *TBC1D1* in the neuronal control of food intake can be excluded, an increased fat oxidation in skeletal muscle is likely causal for the obesity-suppressive effect of *Tbc1d1* deletion. A detailed understanding of the function of *TBC1D1* can provide a new approach for a successful therapy of obesity.

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Effects of frequent weight cycling on longevity in C57BL/6 and 129Sv mice

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Background and aims: The majority of obese patients cannot maintain hypo caloric diets over a longer period of time. As a consequence weight cycling is a frequent phenomenon in long term treatment of obesity. Long-term studies on weight cycling and intentional weight loss suggest that non-pharmacological and non-surgical treatment of obesity might entail elevated mortality rates. Therefore, we tested the hypothesis that weight cycling reduces life span in comparison to both permanent hyper caloric and normal nutrition in two different mouse strains.

Materials and methods: In female 129S6/SvEvTac (N=90) and C57BL/6NTac (N=90) the effects of a continuously caloric restriction, a life-long high fat diet (Sniff, E15772-34) and weight cycling, which was realized with a 4 week period change between both extremes of diet, on longevity have been determined. Body weight was recorded weekly. Intraperitoneal insulin tolerance (ITT) test was performed at the age of 24 and 51 weeks and the HbA1c was analyzed at the age of one year. Basal metabolic rate was measured with metabolic chambers (TSE, Bad Homburg, Germany).

Results: The average life expectancy for weight cycling animals (129S6/SvEvTac average age at death: 21.5±5 month and C57BL/6NTac 21.5±3.5 month) was comparable with the controls under chow diet (129S6/SvEvTac average age at death: 23.5±5 month and C57BL/6NTac 21.1±4 month). The permanent high caloric diet group had a 3.5 month shorter life span than the two other groups (129S6/SvEvTac average age at death: 20.5±5 month and C57BL/6NTac 17.1±4.8 month). 129S6/SvEvTac mice lived 1.5 months longer than C57BL/6NTac mice (*P*<0.0001). In addition, significant differences be-

tween the dietary regimens were found for measures of insulin sensitivity (ITT), glucose metabolism (HbA1c) and energy expenditure.

Conclusion: Frequent weight cycling significantly improved longevity compared to a lifelong high fat diet. There was no difference in the life span of mice undergoing weight cycling and permanent chow diet.

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ASP-C5L2 pathway disruption influences the development of insulin resistance

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Background and aims: Acylation Stimulating Protein (ASP) is an adipose tissue secreted hormone that derives from the immune system. ASP positively contributes to lipid and glucose uptake in adipocytes, increasing triglyceride storage. ASP acts through the C5L2 receptor, found in adipocytes and also in muscle, liver, brain and several immune cells. The ASP-C5L2 pathway is implicated in energy expenditure and has been linked with systemic inflammation. C5L2-deficient mice (C5L2KO) show an obesity-resistant phenotype, conferring a therapeutic potential to the disruption of the ASP-C5L2 pathway. However, previous studies suggest that ASP could also play a role in the development of insulin resistance. This study examines in details energetic metabolism and chronic inflammation alterations in C5L2KO mice with respect to insulin resistance progression.

Materials and methods: We used a diabetogenic regimen (high-fat/high-sucrose diet) for 12-week in C5L2KO and wild type (WT) mice. We performed glucose and insulin tolerance tests, injected radioactive tracers and assessed tissue and plasma metabolic, genetic and inflammatory parameters in all mice.

Results: Our work shows an exaggerated response with increased insulin resistance in C5L2KO mice compared to WT animals when fed a diabetogenic diet. Similar glucose clearance is achieved in C5L2KO mice only with vastly superior insulin levels (*p*<0.001) while a steady bolus of insulin failed to reduce blood glucose in C5L2KO mice as much as in WT mice (*p*<0.05). In addition, we demonstrated that C5L2KO mice show a significant shift in metabolic fuel usage in major organs related to energy expenditure and storage. Glucose uptake was reduced by more than 50% in adipose tissue and skeletal muscle while increased by 30% in liver (*p*<0.05 for all). We also show evidence of a chronic proinflammatory phenotype in C5L2KO mice, linked with a rise in circulating cytokines (such as IL6, IL10, KC and MIP-1), as well as increased macrophages infiltration and polarization in the adipose tissue.

Conclusion: Our study suggests that the ASP-C5L2 pathway may play an important role in the development of obesity co-morbidities such as type 2 diabetes through an energetic metabolism-immune system synergy.

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A maternal high fat diet modulates energy intake in adult offspring in Gpr1/- mice

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Background and aims: Evidence from a variety of animal studies indicates that maternal high fat diet (HFD) consumption programs offspring for increased risk of adult obesity, hyperphagia and increased preference for fatty foods. Among potential mechanisms for perinatally applied HFD induced programming of obesity and hyperphagia are elevated inflammatory cytokines, since many neural systems that are critical in regulating energy balance are sensitive to circulating cytokine levels. There is also a line of evidence that inhibition of GIP signaling prevents the onset of HFD induced obesity. Little is known about the impact of consuming a HFD in adulthood in offspring that were exposed to a HFD during pregnancy and lactation (IU/L), which represents the western lifestyle more realistically than solely exposing mice to a HFD during IU/L. Up to now it is unclear if a HFD during IU/L can program alterations in adiposity or food intake in mice which lack GIP receptor signalling. We hypothesize that GIP receptor knock out (Gipr^{-/-}) mice are protected from dysregulated energy balance due to HFD applied during IU/L.

Materials and methods: Female GIP receptor heterozygous (Gipr^{+/-}) mice on a C57Bl/6J background had ad libitum access to either HFD (60% fat, HFD IU/L) or control diet (CD, 10% fat, CD IU/L) for 2 weeks prior to mating with Gipr^{+/-} male mice and during pregnancy and lactation. Male Gipr^{+/-} and wild type (WT) offspring were maintained on a normal rodent chow for 22 weeks. Then, mice were fed the same HFD used during the IU/L period for further 20 weeks. Body weight and food intake were determined weekly. At the end of the study, when mice were at the age of 45 weeks, expression of cytokines (MCP1, MIP1alpha) and adiponectin in epididymal adipose tissue were measured by quantitative RT-PCR. HPRT was used as a normalizing gene.

Results: No significant differences in body weight and food intake between WT and Gipr^{+/-} mice, which were exposed to either HFD IU/L or CD IU/L, were observed during the first 22 weeks on normal chow. Re-exposure to HFD in adulthood significantly increased body weight at the age of 45 weeks in WT on a CD IU/L compared to Gipr^{+/-} on a CD IU/L (50.61 ± 0.88 g vs. 43.98 ± 0.93 g, respectively, $p < 0.01$). Although cumulative energy intake (Kcal/BW) increased in WT on a HFD IU/L compared to WT on a CD IU/L, food consumption in Gipr^{+/-} on a HFD IU/L was significantly reduced compared to Gipr^{+/-} on a CD IU/L (39.76 ± 0.71 vs. 44.17 ± 1.04 kcal/BW, respectively, $p < 0.05$). The interaction of diet (IU) and genotype in food intake was significant ($p < 0.01$). The reduced adiposity in Gipr^{+/-} mice on CD and HFD IU/L compared WT mice on a CD IU/L was associated with a marked increase in adiponectin expression level and a significant reduction in a sub-class of inflammatory cytokines (MCP1, MIP1alpha) in epididymal adipose tissue.

Conclusion: The decreased food intake in Gipr^{+/-} on HFD IU/L mice is an unexpected and novel result. Reduced adiposity and inflammatory cytokine expression induced by inhibition of GIP signaling may play a role in producing long-term decrease in food intake even after re-exposure to HFD in adulthood.

Supported by: DFG

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Does rimonabant independently affect NEFA and glucose metabolism?

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Background and aims: Endocannabinoid receptor 1 blockade is proposed to improve metabolic complications of obesity via reduction of food intake (CNS mechanisms) and peripheral effects on adipose, liver and muscle. This sub-study of the Visceral fat reduction assessed by CT scan On Rimonabant (VICTORIA) trial directly compared a lifestyle weight loss program with rimonabant 20mg/day versus placebo on insulin regulation of non-esterified fatty acids (NEFA) and glucose metabolism in vivo.

Materials and methods: Sixty-seven volunteers age 35 to 70 with abdominal obesity and 2 other metabolic syndrome features (excluding diabetes) had measures of total body, visceral and fat free mass (FFM) (DXA; abdominal CT) and 2-step (0.25 and 1.0mU/kg/FFM/min) euglycemic hyperinsulinemic clamps at 0 and 12 months to measure: 1) insulin concentration to suppress plasma NEFA (palmitate) concentration 50% (IC50_{pal}); 2) insulin regulation of glucose disposal (slope of glucose disappearance rate vs. plasma insulin); 3) insulin concentration to inhibit hepatic glucose output 50% (IC50_{HGO}). Complete glucose data for 44 subjects (23 placebo) and NEFA data for 41 (21 placebo).

Results: Percent body fat in both groups fell from baseline (rimonabant 4.5% $p < 0.0001$, placebo 2.1% $p = 0.05$). There was a significant reduction in IC50_{pal} with rimonabant correlating with BMI change ($p = 0.005$, $r = 0.60$), however, this was not significantly different from placebo ($p = 0.5$) when controlling for greater BMI loss. Regulation of glucose disposal improved markedly in both groups ($p = 0.002$, $r = -0.29$) and correlated with visceral fat loss. Although the mean IC50_{HGO} did not change significantly from baseline in either group, intra-individual changes in IC50_{HGO} in the rimonabant group were correlated with changes in visceral fat ($p = 0.006$, $r = 0.42$).

Conclusion: In summary, rimonabant had no additive effect on insulin regulation of NEFA beyond that explained by fat loss alone; intra-individual changes in insulin regulation of hepatic glucose output were correlated with changes in visceral fat and insulin regulation of NEFA in the rimonabant group; insulin regulation of glucose disposal corresponded with visceral fat loss.

Clinical Trial Registration Number: PM-C-0172

Supported by: sanofi-aventis

PS 047 Consequences of obesity

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Serum nitrotyrosine in metabolically obese normal weight individuals

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Background and aims: An association between obesity, inflammation and endothelial dysfunction is known. Oxidative stress & nitrosative cell damage are said to mediate these effects. Free radicals such as superoxide lead to the generation of a potent oxidant called peroxynitrite. Nitrotyrosine (NT), measured in circulation, is a surrogate marker of this process. Normal weight obesity is defined as a high body fat percent (BFP) ($>20\%$ for men, $>30\%$ for women) with normal body weight (BMI <25 kg/m²). This group seems to have an equally high risk for metabolic diseases as that of a frankly obese group. We hypothesize that this group has increased oxidative stress which could be manifested as raised NT levels. In this study, we aim to compare serum NT levels in normal weight (NW), obese (O) & Normal weight Obese (NWO) individuals.

Materials and methods: 150 non diabetic, non hypertensive healthy adults (>18 years) were randomly selected & divided into 3 groups - NW, O, NWO. Anthropometry including body composition (Body mass index, Body Fat Percentage, Waist Circumference, Fat free mass, Waist Hip Ratio) was assessed using Bioelectric Impedance Analysis-Inbody230. The serum NT (ELISA - BIOXYTECH Enzyme Immunoassay for Nitrotyrosine) & high sensitivity C-reactive protein (HsCRP) were assessed.

Results:

OBESITY →	Normal weight n = 14	Normal weight Obese n = 28	Obese n = 34	Normal weight n = 8	Normal weight Obese n = 17	Obese n = 49
	Males			Females		
Sex						
Mean Age	43.1	48.8	47.1	47.4	47.2	46.9
MEAN Waist Circumference	70.5	88.5	93.7	71.4	76.7	89.2
MEAN BMI	19.05	25.2	27.5	20.2	22.3	28.7
MEAN BODY FAT PERCENTAGE	14.5	30.1	32.35	26.7	36.6	45.1
MEAN FAT FREE MASS	45.0	47.86	52.4	35.07	33.5	36.8
NITROTYROSINE ng/ml	1.15	1.8	1.18	0.315	6.95	4.94
HsCRPmg/dl	0.66	1.28	1.76	0.68	1.34	3.53

1. There is a significant difference in BFP among NWO & NO groups. This difference is seen in both males ($p < 0.0001$) & females ($p < 0.0001$).
2. NT levels are higher in NWO group as compared to NW group in both males & females.
3. NT levels were significantly higher in female NWO compared to male NWO group ($p = 0.005$).
4. The BFP is significantly higher in the female NWO group when compared to male NWO group ($p = 0.0002$).
5. HsCRP progressively increases across the 3 groups NW, NWO, & O.
6. NT levels in the NWO group were higher than those in O group.

Conclusion:

1. The significantly higher BFP in NWO groups justifies its classification. The NWO group has a higher risk of oxidative stress as measured by Nitrotyrosine than NW group. Hence NWO group is also a target for preventive strategies.
2. Females are more prone to oxidative stress than males probably because of their sedentary life style, higher visceral fat & poor exercise practices due to social circumstances in a developing country like India.
3. HsCRP, a marker of inflammation, progressively increases in NW, NWO & O groups. However NT level is maximum in the NWO group. Probably, the increased ratio of Fat Free mass Vs Fat mass in the obese Individuals makes these individuals comparatively less prone to oxidative stress when compared to NWO group that have high fat percentage & low skeletal muscle mass.

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Metabolic consequences of obesity in a rural setting of BangladeshA. Akhter¹, M.S. Flora², K. Fatema³, L. Ali³;¹Department of Epidemiology, Bangladesh Institute of Health Sciences (BIHS), ²Department of Epidemiology, National Institute of Preventive and Social Medicine (NIPSOM), ³Department of Biochemistry and Cell Biology, Bangladesh Institute of Health Sciences (BIHS), Dhaka, Bangladesh.

Background and aims: The high prevalence of diabetes, hypertension and dyslipidemia are the manifestations of a pattern of metabolic disturbances related to obesity which has not received due attention in many developing countries. This study aims to describe the link of these manifestations to obesity in a rural Bangladeshi adult population, where conventionally obesity is thought to be a less important problem and so as to inform policy and lay the ground for surveillance interventions.

Materials and methods: This population based cross-sectional study was conducted through screening in camp settings in remote rural areas of Northern Bangladesh, which included a total of 836 participants (468 male and 368 female), aged at or above 30 years. Sociodemographic information was collected through semi-structured data collection form. Anthropometric (BMI and WHR) and clinical data were collected by standardized techniques. WHO guideline for Asian population were used to identify obesity with BMI 18.5–22.9 kg/m² as normal, 23.0–24.9 kg/m² overweight and above 25.0 kg/m² as obese. Central obesity was evaluated by WHR, with cut-off points taken at 0.8 for females and 0.9 for males. OGTT and lipid profile were also preformed. Glucose (fasting and 2 hr after glucose) was measured by glucose oxidase method, total cholesterol, triglyceride and HDL were analyzed by enzymatic-colorimetric method and LDL was estimated by Friedewald's formula. Logistic regression analysis was used with adjustment for potential confounders.

Results: The mean (\pm SD) age (years) of the participants was 46 (\pm 12), BMI (kg/m²) 22.1 (\pm 3.4) and WHR was 0.92 (\pm 0.06). As per criteria followed for Asian population about 38.5% (95% CI 35.2–41.8) of the participants had BMI in the overweight (18.4%) to obese (20.1%) range and 52.7% (95% CI 49.3–56.1) having high risk considering WHR. There was a significant female preponderance in both cases ($p < 0.04$ to 0.02) compared to males. Proportion of diabetes, prediabetes, hypertension and dyslipidemia among this population were 7.2% (95% CI 5.4–9.0), 6.5% (95% CI 4.8–8.2), 25.4% (95% CI 22.5–28.3) and 51.6% (95% CI 48.3–54.9), respectively. Influences of obesity were observed on glycemic status ($\chi^2 < 0.001$), hypertension ($\chi^2 = 0.024$) and dyslipidemia ($\chi^2 = 0.037$). After adjustment for socio-demographic variables, effect on lipid level disappeared. Diabetics (OR, 2.83 with 95% CI, 1.52–5.41) and hypertensives (OR, 1.96 with 95% CI, 1.17–4.63) were about 3 and 2 times, respectively, more likely to be obese in this population.

Conclusions: The data suggest that, an alarming proportion of adult Bangladeshi population have generalized as well as central obesity. Obesity is widely prevalent and may represent a silent epidemic even in this rural population. There is an urgent need for strategies and programs to prevent and control obesity, and thus reduce the metabolic burden in the rural population of Bangladesh.

Supported by: Diabetic Association of Bangladesh

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Discriminant features of “metabolically healthy obese” and “metabolically obese normal weight” phenotypes in healthy postmenopausal womenM. Peppas¹, C. Koliaki¹, E. Boutati², E. Garoflos¹, A. Papaefstathiou¹, N. Katsilambros³, S.A. Raptis^{2,4}, G. Dimitriadis², D. Hadjidakis^{1,2};¹Endocrine Unit, Second Department of Internal Medicine-Propaedeutic, Research Institute and Diabetes Center, Attikon University Hospital,²Second Department of Internal Medicine-Propaedeutic, Research Institute and Diabetes Center, Attikon University Hospital, ³Evgenidion Hospital,⁴Hellenic National Diabetes Center for the Prevention, Research and Treatment of Diabetes Mellitus and its Complications (H.N.D.C.), Athens, Greece.

Background and aims: Although obesity is generally associated with increased cardiovascular risk, not all obese individuals exhibit cardiometabolic abnormalities and not all normal-weight subjects are protected against cardiometabolic disease. This allows classification into the following phenotypes: Metabolically Healthy (MHO) and Unhealthy (MUO) Obese, Metabolically Healthy (MHNW) and Obese (MONW) Normal Weight. Recent research has

focused on potential factors that could predict the above subtypes. Aim of the present study was to evaluate the specific patterns of body composition and fat distribution in all these phenotypes, and their association with blood pressure and indices of obesity, inflammation and insulin resistance, in healthy postmenopausal women.

Materials and methods: A cross-sectional study was performed in 150 postmenopausal women (age 54 ± 7 years, BMI 29.6 ± 5.8 Kg/m²). Based on a detailed 7-item cardiometabolic risk score, normal-weight women were subdivided into MONW ($n=38$) and MHNW ($n=22$), if they had ≥ 2 or 0–1 cardiometabolic abnormalities, respectively. Accordingly, obese women were classified into MHO ($n=22$) and MUO ($n=68$). Physical activity status, blood pressure and a full biochemical profile were evaluated, including high-sensitivity C-reactive protein (hs-CRP) measured by nephelometry. Body composition and fat distribution were evaluated by DXA (Hologic QDR Discovery).

Results: MONW women displayed significantly longer duration of menopause, higher waist circumference, blood pressure, fasting glucose, insulin, total and LDL cholesterol, hepatic aminotransferases, fibrinogen and hs-CRP levels, compared to MHNW ($p < 0.05$). Moreover, they exhibited higher height-adjusted lean body mass, and central adiposity indices, including trunk, abdominal and thoracic fat, as well as abdominal/gluteofemoral and trunk/legs fat ratio ($p < 0.05$). Similar but more pronounced differences were observed in the MUO, compared to MHO. Multivariate binary logistic regression analysis, revealed that duration of menopause ($p=0.05$), trunk/legs ($p=0.04$) and abdominal/gluteofemoral fat ratio ($p=0.005$) could classify correctly 78% of normal-weight and obese women into metabolically healthy and unhealthy phenotypes.

Conclusion: The “Metabolically obese phenotype” exists both in normal-weight and obese postmenopausal women and is associated with hypertension, insulin resistance, dyslipidaemia, inflammation and abnormal fat distribution. DXA-derived parameters, such as abdominal/gluteofemoral and trunk/legs fat ratio, can identify abnormal phenotypes and may serve as predictors of cardiometabolic risk in postmenopausal women.

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Effect of abdominal weight gain and weight loss on insulin sensitivity and insulin secretion in adults who maintain normal glucose regulation during a 5-year periodK. Færch¹, D. Vistisen¹, T. Jørgensen², D.R. Witte¹;¹Steno Diabetes Center, Gentofte, Denmark, ²Research Centre for Prevention and Health, Glostrup, Denmark.

Background and aims: To improve prevention of type 2 diabetes it is important to understand how glucose homeostasis is regulated in healthy individuals. We studied how insulin sensitivity as well as absolute and relative insulin secretion changed in response to abdominal weight gain and abdominal weight loss in individuals maintaining normal glucose regulation during a 5-year period.

Materials and methods: From the population-based, non-pharmacological intervention study Inter99 we included 2,514 participants with HbA_{1c} $< 6.0\%$ both at baseline and after 5 years. All were classified as having either increased or decreased their waist circumference (WC) during the 5-year period. Participants who increased ($n=1,820$) and decreased ($n=694$) their WC were further divided into tertiles, resulting in 6 different groups (small, medium and large WC increase, and small medium and large WC decrease). From OGTTs performed at baseline and after 5 years we estimated insulin sensitivity, absolute early insulin secretion as well as relative beta cell function (i.e. the disposition index).

Results: Estimates of insulin sensitivity and insulin secretion were standardized before analysis. Differences between groups of WC change were tested in linear regression models adjusted for age, sex, intervention group, 5-year change in HbA_{1c}, baseline WC, and baseline variables of insulin sensitivity, absolute or relative insulin secretion where relevant. Changes in WC were associated with changes in insulin sensitivity in a dose-dependent manner (P for trend < 0.001 ; Figure 1). The pattern of absolute insulin secretion was somewhat different (P for trend = 0.127). The increase in absolute insulin secretion was highest in participants with the largest increase in WC ($P < 0.001$), whereas the change in absolute insulin secretion did not differ between the groups with small, medium and large abdominal weight loss ($P \geq 0.279$). The change in disposition index did not differ between the three groups who gained abdominal weight ($P \geq 0.487$), but the disposition index improved more in participants with a large abdominal weight loss compared to participants with a small abdominal weight loss ($P=0.012$).

Conclusion: The changes in insulin sensitivity and insulin secretion in response to abdominal weight gain and abdominal weight loss are not similar. Therefore, it cannot be assumed that the adverse effects observed after abdominal weight gain are restored in response to abdominal weight loss.

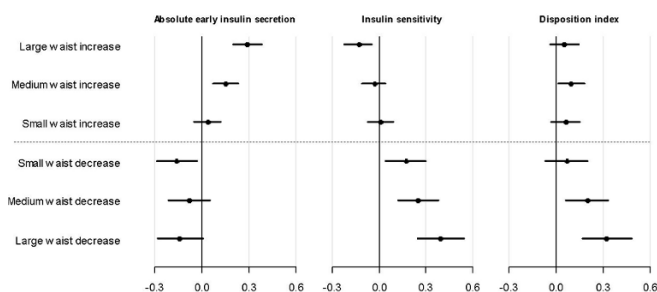


Figure 1: Unadjusted differences (SD) in absolute early insulin secretion, insulin sensitivity and disposition index in persons with small, medium and large waist increase (upper panel) and persons with small, medium and large waist decrease (lower panel).

Clinical Trial Registration Number: KA 98 155

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The association between body fat and circulating complement C3 is largely explained by low-grade inflammation and insulin resistance: the CODAM study

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Background and aims: Serum complement factor 3 (C3) has been linked to type 2 diabetes mellitus and cardiovascular diseases. C3 levels are closely related to body fat, but the underlying mechanisms explaining this association are still unknown. The present, cross-sectional study investigated the role of low-grade inflammation and insulin resistance in adiposity-related increases in C3, since both are known correlates of adiposity and C3.

Materials and methods: Adiposity measures (including waist circumference (WC), body mass index (BMI), sagittal diameter and several skinfolds), serum C3, HOMA2-IR and markers of inflammation (hs-CRP, IL-6, SAA, haptoglobin, ceruloplasmin, sICAM-1) were determined in 534 individuals (62% men, age 59±6.9 yrs) from the CODAM study. Markers of inflammation were standardized and compiled into an average inflammation score. Associations between adiposity measures and C3 were analysed with linear regression models, and multiple mediation analyses were performed to ascertain the extent to which any such associations were explained by inflammation and/or HOMA2-IR.

Results: Adiposity measurements were significantly associated with C3 levels, with the strongest (adjusted) associations found for WC ($\beta=0.385$; 95%CI 0.305-0.466) and sagittal diameter ($\beta=0.413$; 95%CI 0.335-0.491). Further adjustment for inflammation and HOMA2-IR attenuated these associations to $\beta=0.119$ (95%CI 0.034-0.204) and $\beta=0.167$ (95%CI 0.086-0.248) respectively. Multiple mediation analyses showed that inflammation [$\beta=0.091$ (95%CI 0.060-0.127)] and HOMA2-IR [$\beta=0.176$ (95%CI 0.126-0.229)] each explained, independently of one another, a significant portion of the association between WC and C3 (24% and 46%, respectively). Similar mediation by inflammation (19-28%) and HOMA2-IR (36-55%) was found for other adiposity measures (Fig. 1).

Conclusion: Systemic low-grade inflammation and insulin resistance seem to represent two independent pathways by which body fat leads to elevated C3 levels.

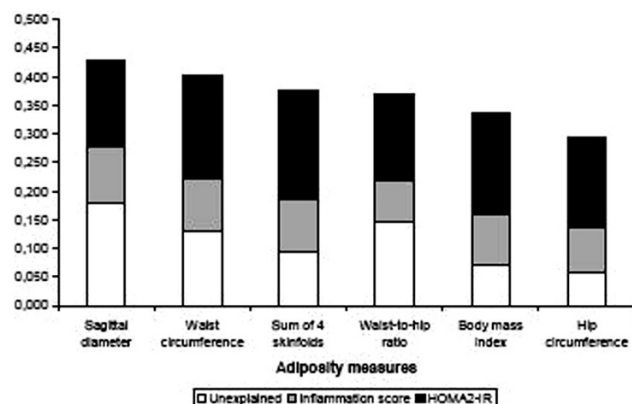


Figure 1: Associations between adiposity measures and serum C3, ordered from strongest to weakest; portions of the overall associations independently explained by low-grade inflammation are given in grey (19-28%) and by HOMA2-IR are given in black (36-55%). Portions that remained unexplained are given in white.

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Insulin sensitivity, beta cell function and subclinical inflammation in the baboon, a non human primate model of insulin resistance, obesity and type 2 diabetes

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Background and aims: Insulin resistance and beta cell dysfunction are hallmarks of type 2 diabetes mellitus (T2DM) in humans. They are early findings of impaired glucose homeostasis in obesity. Previously we have characterized the metabolic and insulin signaling defects in target tissues in nondiabetic baboons. Obesity and insulin resistance occur naturally in baboons and may be associated to low grade systemic inflammation. Here we sought to assess beta cell function and insulin sensitivity in baboons, and the relationship between circulating cytokines with glucose levels and anthropometric measures of adiposity.

Materials and methods: We studied 46 nondiabetic baboons in basal conditions, in which we measured anthropometric measures of adiposity such as waist circumference, BMI and percent body fat (%BF) by DXA, and performed a two step hyperglycemic clamp with arginine stimulus to assess both beta cell function and insulin sensitivity. Partial correlations were performed between insulin sensitivity (M/I), acute insulin response (AIR_{0-12}), second phase insulin (AUC_{12-180}) secretion and arginine potentiated insulin secretion ($AUC_{Arg_{180-210}}$), in addition to body composition. On a separate cohort of animals (n=80) ranging the whole span of adiposity representative of animals living in wild conditions, we measured circulating sTNF-R1 and sIL6-R levels in order to correlate them with glucose metabolism and adiposity.

Results: Waist circumference was strongly correlated with total percent body fat ($r^2=0.72$ $p<0.0001$) and trunk fat ($r^2=0.41$, $p<0.01$) in our study population. Waist circumference, BMI, and total body weight showed a markedly gender dimorphism although the strong relationship with percent body fat was maintained in both males and females. During the hyperglycemic clamp, M/I was negatively correlated with total insulin secretion ($r^2=0.70$, $p<$), AIR_{0-12} ($r^2=0.12$, $p=$), 2nd phase ISEC₀₋₁₈₀ and ISEC_{ARG} (both $r^2=0.66$, $p<0.0001$) during each stage of the clamp and with the ratio between insulin and glucagon secretion ($r^2=0.30$, $p<0.0001$). M/I did not correlate with glucagon secretion in the clamp. Finally, sTNF-R1 correlated with fasting plasma glucose ($r^2=0.19$, $p<0.0001$). Weight showed a correlation with sIL6-R ($r^2=0.09$, $p<0.004$), sTNF-R1 levels correlated with sIL6-R ($r^2=0.10$, $p=0.003$).

Conclusion: Insulin secretion during the hyperglycemic clamp correlated inversely with insulin sensitivity in baboons in the nondiabetic range. Also, progressive increase in fasting plasma glucose in the nondiabetic range was directly correlated to an increase in circulating markers of subclinical inflam-

mation known to be strongly correlated with insulin resistance in humans by dysregulation of the TIMP-3/TACE dyad, with increased production of TNF- α and IL6. These results further establish the use of baboons as a valuable non human primate model for the study of complex metabolic diseases such as obesity, insulin resistance and T2DM.

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Lower weight gain and better outcomes in patients with type 2 diabetes starting insulin treatment when baseline HbA_{1c} <8.0%

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Background and aims: Weight gain is a commonly perceived effect of using insulin in type 2 diabetes (T2D).

Materials and methods: We examined weight gain in the context of treatment efficacy and safety using pooled data from 9 randomized, controlled, clinical studies of adult patients with T2D. In each study, insulin glargine was tested against a comparator (63% other insulins, 32% OADs, 6% dietary) over 24 weeks. Weight gain was assessed by treatment, demographics, age, and baseline A1C and FPG. The analysis included 2900 patients (Glargine: n=1449; Comparator: n=1451).

Results: Patients were 56% male, 84% white, mean (SD) age 57 (9.8) years, and T2D duration 8.6 (6.1) years. Mean weight gain was similar for Glargine-treated patients vs Comparator (2.2 kg vs 2.1 kg). However weight gain was lowest in patients with baseline A1C <8%, rising with higher A1C. Results were similar for baseline FPG. Also patients ≥ 65 years gained less weight during treatment than younger patients and weight gain was significantly lower as a patient's age increased. More patients achieved A1C $\leq 7\%$ with Glargine (58.3%) vs Comparator (52.7%) (OR=1.27, $P=0.0015$). Trend analysis showed older vs younger patients treated with Glargine were more likely to achieve A1C $\leq 7\%$ ($P=0.0055$); there was no such trend for Comparator. Glucose confirmed (<50 mg/dL) hypoglycemia occurred less often with Glargine treatment than Comparator, 1.5 vs 2.6 events/pt-yr, $P<0.0001$, with the lowest estimate among Glargine patients ≥ 65 years.

Conclusion: Weight gain with Glargine therapy is similar to that for Comparator, but varies with patients' demographics. Starting patients when A1C <8% leads to better efficacy, comparable safety, and minimizes weight gain. Also patients ≥ 65 years show the lowest weight gain and highest odds of achieving A1C $\leq 7\%$ with least hypoglycemia.

Weight gain by treatment, baseline A1C and age

	Baseline		Week 24		Change from Baseline	
	Glargine Mean (SD)	Comparator Mean (SD)	Glargine Mean (SD)	Comparator Mean (SD)	Glargine Mean (SD)	Comparator Mean (SD)
Aged <50 years	96.9 (19.8)	98.3 (21.6)	99.9 (20.4)	101.2 (22.4)	3.0 (4.5)	2.9 (4.6)
Aged ≥ 50 to <65 years	91.1 (17.5)	91.0 (17.3)	93.2 (18.3)	93.1 (18.1)	2.1 (3.6)	2.1 (3.9)
Aged ≥ 65 years	85.0 (14.8)	84.3 (14.5)	86.5 (15.0)	85.8 (15.0)	1.5 (3.4)	1.5 (3.5)
Overall	90.9 (17.9)	91.2 (18.4)	93.1 (18.6)	93.3 (19.2)	2.2 (3.8)	2.1 (4.0)
Pearson correlation (P-value)					-0.163 (<.0001)	-0.122 (<.0001)
Baseline A1C <8%	89.5 (15.5)	89.7 (17.5)	90.8 (16.1)	90.8 (18.3)	1.3 (3.2)	1.0 (3.9)
Baseline A1C $\geq 8\%$ to 9%	90.0 (17.5)	91.1 (17.4)	92.8 (18.1)	93.2 (18.2)	2.0 (3.6)	2.1 (3.9)
Baseline A1C $\geq 9\%$	91.9 (19.6)	92.3 (20.0)	94.9 (20.4)	95.2 (20.7)	2.9 (4.1)	3.0 (4.0)
Overall	90.9 (17.9)	91.2 (18.4)	93.1 (18.6)	93.3 (19.2)	2.2 (3.8)	2.1 (4.0)
Pearson correlation (P-value)					0.195 (<.0001)	0.241 (<.0001)

Supported by: Sanofi-Aventis, US

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Fat distribution, HbA_{1c} and hypoglycaemia in overweight and obese patients with type 2 diabetes: a comparison of NPH and insulin detemir from a pilot randomised clinical trial

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Background and aims: Weight gain and hypoglycaemia remain significant barriers to insulin intensification in type 2 diabetes (T2D), and the former may be of particular concern to patients who are overweight. Insulin detemir (IDet) has been shown to incur less weight gain than other basal insulins, which may facilitate greater patient acceptance of insulin.

Materials and methods: This was a 26-week, open-label, randomised trial designed to compare the effects of IDet and NPH insulin on weight parameters in overweight or obese patients with T2D. The primary endpoint was change in trunk fat mass, measured by double energy X-ray absorptiometry (DEXA). Secondary endpoints included weight change (kg), waist circumference, BMI, HbA_{1c} (%) and hypoglycaemia. Both basal insulins were administered once daily, with insulin aspart given at main meals. Patients taking OADs with the exception of metformin were excluded.

Results: Demographic characteristics were similar at baseline for IDet (n=24, mean age 60.6 years, 54.2% male, mean HbA_{1c} 8.3 %, mean BMI 32.2 kg/m², mean waist circumference 107.7 cm) and NPH insulin (n=35, mean age 63.7 years, 45.7% male, mean HbA_{1c} 8.3 %, mean BMI 34.0 kg/m², mean waist circumference 110.0 cm). Overall, changes in trunk fat mass, whole-body fat mass and weight were similar between the two groups, as was dose and glycaemic control, with a favourable trend towards IDet. IDet incurred a statistically significant lower risk of hypoglycaemia (see table). Differences were also noted for change in waist circumference at week 8 (-1.05 cm [5.21 SD, IDet group] vs. 1.34 cm [3.67 SD, NPH insulin group], $p=0.0173$) and week 12 (-0.9 cm [4.90 SD, IDet group] vs. 1.04 cm [3.47 SD, NPH insulin group], $p=0.045$), but these were not maintained at study end.

Conclusion: In summary, compared with NPH insulin treatment, IDet seems to result in a small reduction in waist circumference, a similar reduction in HbA_{1c} and significantly fewer episodes of hypoglycaemia.

Change from baseline at 26 weeks

	IDet (n=24)	NPH insulin (n=35)
Trunk fat mass (% change)	-0.38% (10.97)	-0.06% (12.57)
Whole-body fat mass (% change)	2.59% (12.39)	2.59% (12.39)
Weight change (kg)	0.89 kg (4.30)	1.90 kg (2.79)
HbA _{1c} reduction (%)	-0.79% (1.06)	-0.73% (1.16)
Number of hypoglycaemia events	6.42 (9.45)*	15.31 (20.90)
Daily basal insulin dose (U/kg)	0.41 (0.14)	0.47 (0.17)

Data are given as mean (SD); * $p<0.05$ in favour of insulin detemir (IDet)

Clinical Trial Registration Number: NN304-1677

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Insulin upregulates natriuretic peptide clearance receptor expression in the subcutaneous fat depot in obese subjects: a missing link between CVD risk and obesity?

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Background and aims: Natriuretic peptides (NP) have multiple metabolic properties and NP concentrations have been shown to be down regulated in patients with central obesity. NP act via binding on three natriuretic peptide receptors (NPRs). Increased expression of natriuretic peptide clearance receptor (NPRC) in subcutaneous adipose tissue was observed in central obesity. We therefore tested hypothesis that insulin may regulate NPR expression in the fat tissue.

Materials and methods: NPR (A-C) mRNA expression was measured in paired samples of visceral (VAT) and subcutaneous adipose tissue (SAT) from 157 subjects with (n=72) or without (n=85) type 2 diabetes. The effect of insulin on NPR genes expression in SAT was studied in euglycemic-hyperinsulinemic and hyperglycemic-hyperinsulinemic clamp experiments in obese male subjects (n=14). Additionally, the effect of insulin and glucose on NPR expression in culture of primary human monocytes and macrophages was tested.

Results: NPRA and NPROC gene expression were higher in VAT compared with SAT ($p<0.01$), but only NPROC gene expression strongly correlated with fasting insulin levels ($r=0.65$, $p=0.04 \times 10^{-3}$ and $r=0.54$, $p=0.002$, for VAT and SAT respectively). NPROC gene expression was up-regulated in both euglycemic- and hyperglycemic-hyperinsulinemic clamps ($p=0.038$ and $p=0.048$, respectively). NPROC expression was increased in simultaneous insulin and glucose treatment in monocytes (70.2%, $p=0.01$), but not in mature macrophages.

Conclusion: Insulin increases expression of NPROC in SAT independently of glucose concentration. Thus insulin might suppress levels of circulating NP via upregulation of NPROC expression in obesity. Our observations provide a novel link between hyperinsulinemia, cardiovascular disease risk and the metabolic syndrome.

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PS 048 Consequences of high fat diet

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Influence of the high-fat diet on the circadian clock in human peripheral mononuclear blood cells

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Background and aims: The circadian clock coordinates various behavioral and physiological processes including feeding behavior and energy metabolism. In turn, metabolic processes feed back onto the circadian clock as shown in recent studies of obesity and type 2 diabetes. Moreover, animal studies demonstrated alterations of behavioral and molecular circadian rhythms induced by the high-fat diet. However, there is little to no information about the effect of nutritional components on circadian mechanisms in humans. To address this, we investigated the influence of a high-fat isocaloric diet on daily expression profiles of clock genes in human peripheral mononuclear blood cells (PBMC) and purified monocytes.

Materials and methods: The expression of 10 clock genes was determined by real-time PCR in twelve nonobese healthy individuals in terms of NUGAT (Nutrigenomics Analysis in Twins) study. Gene expression was measured in three time points (at 8:30, 11:45 and 16:00) during three investigation days. The blood sampling was carried out before the beginning of the high-fat isocaloric diet (45 % kcal from fat) and after one and six weeks of intervention.

Results: We firstly demonstrated that Per1, Per2, Per3, Bmal1, Rev-erba, Dbp and Tef are rhythmically expressed in human monocytes. The clock genes Clock, Cry1 and Cry 2 displayed no significant circadian rhythmic. In PBMC, similar temporal expression profiles were detected. In response to high-fat diet, in monocytes, the morning expression and amplitude of the Period genes Per1, Per2 and Per3 increased after one and/or six weeks of intervention and correlation analysis reveals stronger relationship between clock genes after six weeks of diet. In contrast, in PBMC fraction, we found no significant changes of clock gene expression and modest decrease of synchronisation of the clock gene mechanism after six weeks of diet intervention.

Conclusion: High-fat diet induces alterations of clock gene expression in human monocytes which are undetectable in heterogenic PBMC population. Therefore, the investigation of the purified monocytes as metabolic important immune cell fraction is of interest in human studies. Our results suggest that the consumption of a high-fat isocaloric diet can influence the circadian mechanism in human already after the short time intervention and emphasize the role of nutrition-clock interaction in the regulation of human metabolism.

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Chemerin and chemokine-like receptor 1 in mice with high fat diet induced obesity

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Background and aims: Chemerin is a recently discovered protein predominantly produced in the liver and adipose tissue. It has been shown to influence adipocyte differentiation and mediate chemotaxis as well as activation of dendritic cells and macrophages. These functions are partially mediated via chemokine-like receptor 1 (CMKLR1). Several studies suggested negative associations between chemerin and glucose tolerance. In humans, circulating chemerin levels decrease with significant weight loss after bariatric surgery, suggesting that chemerin might contribute to the negative relationship between obesity and insulin sensitivity.

Materials and methods: In this study we set out to investigate the influence of dietary fat content on circulating chemerin levels and the tissue specific expression patterns of chemerin and CMKLR1 in an animal model of diet-induced obesity. Ten male C57Bl/6 mice were fed either standard diet (fat content 12% kcal) or high fat diet (fat content 60% kcal) for 10 weeks. Circulating chemerin levels were determined by using a commercially available

ELISA. Chemerin as well as CMKLR1 mRNA expression levels in visceral, subcutaneous adipose tissue and liver tissues were quantified by fluorescence based real-time PCR.

Results: As expected body weight was significantly higher in mice fed a high fat diet than in standard diet fed mice (47.3 ± 4.1 g vs. 38.9 ± 1.7 g; $p < 0.01$). Circulating chemerin levels were significantly increased in high fat diet fed mice when compared to standard diet fed mice (109.4 ± 14.1 ng/ml vs. 88.1 ± 10.9 ng/ml; $p = 0.03$). CMKLR1/GAPDH cDNA ratios of visceral and subcutaneous adipose tissue and the liver were unaffected by dietary fat content. Chemerin/GAPDH cDNA ratios in both subcutaneous and visceral adipose tissue were also similar in high fat and standard diet fed mice. Hepatic Chemerin expression tended to be higher in high fat diet fed mice without reaching statistical significance.

Conclusion: In conclusion, circulating chemerin levels are significantly increased in diet-induced obese mice. Elevated circulating Chemerin might not result from increased chemerin expression levels in adipose tissue or the liver but by enlarged mass of adipose tissue in obesity. Our data suggest that chemerin might be another component linking high fat diet and insulin resistance.

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Selective breeding of mice which are prone and resistant to high fat diet-induced obesity and hyperglycaemia

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Background and aims: To elucidate key factors for regulating susceptibility to high fat diet (HFD), we have conducted a selective breeding to establish two strains of mice which exhibit hyperglycemia with obesity and normoglycemia without obesity after 5-week HFD feeding; designated Selectively bred Obese and Diabetes Prone (SOD-P) and Resistant (SOD-R) mice, respectively. We previously demonstrated that SOD-P mice showed significantly higher blood glucose level and gained more body weight through the HFD feeding period compared with SOD-R mice.

Materials and methods: Each strain of mice fed a HFD (30% kcal fat) for 5 weeks (from 5 to 10 weeks of age), and oral glucose tolerance test (OGTT; 2g/kg body weight) was performed after HFD feeding. The mice, which exhibited higher and lower 2-h post-OGTT blood glucose concentration, were respectively selected for SOD-P and SOD-R, and have been bred over the 14th generations so far. To evaluate the characteristics of these selectively bred mice, OGTT and insulin tolerance test (ITT; 1U/kg body weight, i.p.) was performed before and after HFD exposure (at 5 and 10 weeks of age, respectively). Glucose stimulated insulin secretion (GSIS) from pancreatic islets was assessed by batch incubation of isolated islets.

Results: At 5 weeks of age, i.e., even before exposure to HFD, male SOD-P mice gained more body weight (19.9 ± 0.4 g in SOD-P, 18.7 ± 0.4 g in SOD-R; $P = 0.048$) and exhibited modestly higher blood glucose concentrations in OGTT ($P = 0.009$, AUC_{120min}), but similar glucose-stimulated plasma insulin response compared with SOD-R mice. In addition, SOD-P mice showed reduced insulin sensitivity in ITT. After HFD feeding (at 10 weeks of age), body weight of SOD-P and SOD-R was significantly different (32.0 ± 1.3 g and 25.5 ± 1.3 g, respectively; $P = 0.003$), and SOD-P mice showed higher blood glucose response in OGTT ($P = 0.008$, AUC_{120min}) and lower insulin sensitivity in ITT compared with SOD-R mice. There was no difference in plasma insulin response in OGTT. These results indicate that heritable glucose metabolism impairment and obesity in SOD-P mice become more evident by exposure to HFD. At 5 weeks of age, although apparent islet size was not different, GSIS from male SOD-P islets was impaired under high glucose (16.7 mmol/l) condition compared with SOD-R islets.

Conclusion: The present results indicate that HFD-induced weight gain and impaired glucose metabolism as heritable phenotypes were accelerated through the selective breeding study. The difference in susceptibility to HFD would enable us to establish these two strains of mice, SOD-P and SOD-R. The obese and diabetes prone SOD-P mice showed whole body insulin resistance and impaired GSIS in pancreatic islets even before HFD feeding. These factors may be crucial for determining the susceptibility to HFD-induced obesity and diabetes.

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The effects of high-fat diet on intestinal and pancreatic functions with emphasis on perfusion and glucose uptake; validation of the [18 F]FDG radionuclide using simultaneous PET and CT imaging modalities

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Background and aims: Today intestine is regarded as highly active metabolic and endocrine organ. Changes in the secreted enteroendocrine hormone profile are crucial in the pathogenesis of the type 2 diabetes mellitus (T2DM). However, there is no consensus about the changes that precede the altered hormonal secretion; both circulatory dysfunction and altered nutrient uptake of the mucosal cells have been suggested. Our aim was to validate the use of positron emission tomography (PET) on the intestine and pancreas in a pig model and to compare the extents of perfusion (with radiowater, [15 O]H₂O) and glucose uptake (with fluorine-18 labeled glucose analogue [18 F]FDG) between diabetic and healthy swines.

Materials and methods: Twelve age-matched swines were randomized into two groups. Intervention group (n=6) were administrated with streptozotocin + high-fat diet in order to achieve obese and diabetic state. Simultaneous PET/CT was used in perfusion and glucose uptake scans. Multiple arterial plasma samples were collected to obtain plasma radioactivity measurements and metabolic parameters. Tissue samples from three parts of intestine (duodenum, ileum, colon) and pancreas (caput, corpus, cauda) were obtained. Autoradiography, histologic and immunohistochemical sections were prepared and tissue radioactivity was measured. Gjedde-Patlak plot was used to obtain PET derived Ki values.

Results: By the time of writing, most of the swines have been imaged and data obtained although no data-analyses have been executed. However, the following statements can already be made: 1) Autoradiography show that most of the glucose analogue accumulates in the mucosal layer of the intestine. This comprises the most metabolically active part of the intestinal wall. 2) Preliminary data indicate that both the pancreatic and even more intestinal perfusion is decreased in obese diabetic swines (73.5 vs. 54.2 ml/(100g min) for pancreas; 50.3 vs. 37.4 ml/(100g min) for duodenum; 56.1 vs. 35.4 (ml/100g min) for ileum; 21.7 vs. 15.4 (ml/100g min) for colon). In the case of intestine, this can be due to the endothelial dysfunction of the mucosal vessels induced by intervention (high-fat diet) or changes in secreted enteroendocrine profile via dorsal motor nucleus of the vagus (DMV) and splanchnic NANC (non-adrenergic, non-cholinergic) neurons. Histologic sections stained with Movat's pentachrome and glucose and fatty acid transporters enlighten these hypotheses. At this early stage it is not definite to say whether correction coefficient or function is needed between ex vivo and PET derived Ki values.

Conclusion: Our preliminary data show that both intestinal and pancreatic perfusion is decreased in obese diabetic subjects. Moreover, PET can indeed be used for the studies regarding intestine and pancreas. Further studies regarding the specific actions of enteroendocrine hormones on perfusion are needed. With fluorine-18 labeled radionuclide (including glucose and free fatty acid analogues) studies with PET being validated on the intestine and pancreas, a whole new highway for noninvasive metabolic studies will be opened.

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N-3 fatty acids augment beneficial effects of calorie restriction in mice fed a high-fat diet: role of lipid mediators

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Background and aims: Calorie restriction (CR) is an essential component in the treatment of obesity and associated diseases. n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) act as natural hypolipidemics, reduce risk of cardiovascular disease and could prevent development of obesity and insulin resistance. We aimed to characterize the efficacy and underlying mechanisms

of the combination treatment with n-3 LC-PUFA and mild CR in the prevention of obesity and associated disorders in mice.

Materials and methods: Male C57BL/6J mice were habituated to a corn oil-based high-fat diet (cHF) for 2 weeks and then randomly assigned to various dietary treatments for 5 weeks or 15 weeks: (i) cHF, ad libitum; (ii) cHF+F, cHF diet with n-3 LC-PUFA concentrate (EPAX 1050 TG) replacing 15% of dietary lipids, ad libitum; (iii) cHF+CR, cHF diet reduced by 10% as compared with the ad libitum cHF-fed mice; and (iv) cHF+F+CR.

Results: Compared with the cHF diet, the combination treatment (cHF+F+CR) most efficiently reduced body weight gain and the weight of both abdominal and subcutaneous white adipose tissue (WAT) depots, while it also improved insulin sensitivity (HOMA index) and glucose tolerance (OGTT). Ectopic lipid accumulation in the liver and skeletal muscle was also markedly decreased by the combination treatment. Moreover, the infiltration of WAT by macrophages, indicating a chronic low-grade inflammation of the tissue, was prevented by the cHF+F+CR treatment. Specifically in abdominal WAT, the conversion of PUFA to anti-inflammatory lipid mediators was additively induced by CR and n-3 LC-PUFA. For instance, the levels of 15-deoxy- $\Delta^{12,15}$ -prostaglandin J2 and neuroprotectin D1 were increased 6.6-fold and 35-fold, respectively, by the cHF+F+CR treatment as compared with the cHF mice. Furthermore, gene expression analysis revealed synergistic activation of PPAR α /PGC-1 α signalling pathways by the cHF+F+CR treatment in abdominal WAT, but not in the liver, muscle or brown fat. The above effects of the combination treatment correlated with the rate of palmitate oxidation and mitochondrial respiratory capacity in WAT. Thus, the maximal rate of palmitate oxidation measured in epididymal WAT *ex vivo* was 2-fold higher in the cHF+F+CR mice as compared with the cHF mice (60.01 ± 8.04 vs. 24.73 ± 5.67 pmol O $_2$ /s/mg DNA; $p = 0.012$), when measured in the presence of FCCP, an uncoupler of oxidative phosphorylation.

Conclusion: We show that n-3 LC-PUFA augment the anti-inflammatory and metabolic effects of CR through the induction of mitochondrial biogenesis and fatty acid oxidation in WAT, while inducing tissue-specific changes in the production of anti-inflammatory lipid mediators. Thus, this study reveals a new mechanism of anti-inflammatory and metabolic effects of n-3 LC-PUFA through the WAT-specific induction of anti-inflammatory lipid mediators including 15-deoxy- $\Delta^{12,15}$ -prostaglandin J2.

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Bile acid mediated resistance to high-fat diet induced obesity is not mediated through bile acid (TGR5) receptor

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Background and aims: TGR5 has identified as a receptor activated by bile acids and activation of TGR5 receptor stimulates GLP1 and PYY secretion from enteroendocrine cells. Further, chronic exposure to high levels of bile acid leads to weight loss, and this has been suggested to be caused by TGR5 mediated increase of energy expenditure. In this study, we aimed to understand whether bile acid-induced weight loss is due to activation of TGR5 receptor.

Materials and methods: Lean or diet-induced obese C57/BL6 mice were fed by high fat diet alone or high fat diet containing 0.5% cholic acid for the duration of 12 weeks to assess bile acid induced prevention of weight gain or weight loss, respectively. Body weight and food intake were monitored every three days and body composition was measured every two weeks by quantitative nuclear magnetic resonance. Another group of wild-type and TGR5 null mice were also fed by bile acid diet.

Results: Cholic acid significantly prevented weight gain in lean mice throughout the study without affecting food intake. In diet-induced obese mice, cholic acid treatment significantly reduced weight loss (-10%) for up to 6 weeks. However, cholic acid also prevented high fat diet induced weight gain in TGR5 null mice.

Conclusion: These results suggest that the TGR5 receptor is of no importance in mediating bile acid induced resistance to high-fat diets.

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The role of adipose tissue in insulin-sensitising and blood pressure lowering effects of ACE-inhibitors: studies on mice with diet-induced obesity and fatless mice

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Background and aims: Inhibitors of angiotensin-converting enzyme (ACE) not only decrease blood pressure but also improve insulin sensitivity and decrease incidence of diabetes. The aim of our study was to assess the significance of adipose tissue in blood pressure-lowering and metabolic effects of ACE-inhibitor ramipril.

Materials and methods: We studied metabolic and blood pressure-lowering effects of ACE inhibitor ramipril in severely insulin resistant and diabetic lipotrophic A-ZIP/F-1 mice which lack white adipose tissue and in C57BL/6J mice with high fat diet (HFD)-induced obesity and insulin resistance. Blood glucose, circulating levels of insulin and other hormonal parameters were measured by commercial RIA and ELISA kits and mRNA expression of selected adipokines in subcutaneous (SAT) and visceral adipose tissue (VAT) was measured by real time PCR.

Results: Three months of treatment with ramipril (8 mg/kg in the food) did not significantly affect blood glucose, serum insulin, triglycerides or free fatty acids in A-ZIP mice but it partially prevented the increase of body fat content, decreased adipocyte size, free fatty acids (1239.95 ± 97.89 vs 597.88 ± 73.89 μ M, $p < 0.05$) and triglyceride (12.54 ± 1.02 vs 6.79 ± 0.56 mg/dl, $p < 0.05$) levels and improved insulin sensitivity as measured by insulin concentrations (1.73 ± 0.3 vs 0.60 ± 0.1 ng/ml, $p < 0.05$) and HOMA index (0.77 ± 0.12 vs 0.31 ± 0.05 , $p < 0.05$) in HFD-fed C57BL/6J mice. In subcutaneous fat, ramipril treatment prevented a decrease of adiponectin mRNA expression induced by HFD and tended to decrease mRNA expression of macrophage marker Emr1 and TNF- α . In visceral adipose tissue, ramipril prevented a decrease of adiponectin mRNA expression induced by HFD, significantly blunted increased Emr1 and MCP-1 and tended to decrease interleukin-6 and TNF- α mRNA expression. Mean arterial blood pressure (MABP) in A-ZIP mice was significantly higher compared to C57BL/6J mice. Ramipril treatment decreased MABP in both A-ZIP (96 ± 6.0 vs 71 ± 4.0 Torr, $p < 0.05$) and C57BL/6J mice (72 ± 3 vs 62 ± 4 Torr, $p = 0.122$). **Conclusion:** ACE inhibition by ramipril partially prevented the development of obesity and insulin resistance in mice fed HFD while it did not affect insulin sensitivity in fatless A-ZIP mice. In contrast, blood pressure-lowering effect of ramipril was present in both control and fatless A-ZIP mice. We conclude that while insulin-sensitizing effects of ACE-inhibition require the presence of adipose tissue its blood pressure-lowering effects are independent of the presence of fat.

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Dietary trans fatty acids are associated with impaired endothelial function in type 2 diabetic patients, independently of other cardiovascular risk factors

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Background and aims: There has been a growing interest in the role of nutritional factors, especially the fatty acids intake, in modulating endothelial function. Few studies analyze the association between dietary fatty acid composition and vascular function in diabetic patients, and most of them evaluated only their acute effects on endothelial function. This cross-sectional study aimed to evaluate the possible association between dietary fatty acid composition and impairment of endothelial function measured by flow-mediated dilatation (FMD) of type 2 diabetic patients.

Materials and methods: The nutritional evaluation consisted of completing 3-day weighed diet records (WDR). Compliance with the WDR was evaluated by protein intake confirmed by 24-h urinary urea. Clinical evaluation consisted of metabolic and blood pressure control, detection of chronic complications of DM and cardiovascular evaluation. The patients underwent assessment of endothelium-dependent FMD of the brachial artery to evaluate endothelial function.

Results: Eighty-four patients were evaluated (age:63±9 years;64%male) and the observed median endothelium-dependent FMD was 5.55% (1.19–20.10). In multiple linear regression analyses, log-transformed FMD (dependent variable) was inversely associated to dietary trans fatty acids (TFA) intake (β -Standardized Coefficients = -0.387; $P=0.001$; $R^2=0.271$, $P=0.001$), and to the presence of diabetic nephropathy (β -Standardized Coefficients = -0.235; $P=0.04$), adjusting to systolic blood pressure, adequacy of WDR, gender and waist circumference. In a multivariate logistic regression analysis, lower FMD (median FMD < 5.55 %; dependent variable) was positively associated with dietary TFA intake (Odds Ratio (OR)=7.89; 95%Confidence Interval (CI):1.85–33.7; $P=0.005$) and presence of diabetic nephropathy (OR = 4.77; 95%CI:1.37–16.6; $P=0.014$), after adjusting for covariates.

Conclusion: In patients with type 2 diabetes, a higher intake of TFA is inversely associated with endothelial function assessed by ultrasound of the brachial artery, independently of other cardiovascular risk factors.

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PS 049 Lipids and cardiovascular risk

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Nonfasting blood concentration of lipoprotein subfractions and risk of coronary heart disease in a Swedish nested case-control study

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Background and aims: The ion mobility method is unique in its capability for directly determining concentrations of the full spectrum of lipoprotein particles, from small HDL to large VLDL, as a function of their particle size. Several studies have shown significant associations between nonfasting lipid levels and risk of cardiovascular disease, but the evidence of association between nonfasting lipoprotein subfractions and cardiovascular risk is limited. The aim of this project was therefore to examine the association between plasma concentration of lipoprotein subfractions and risk of coronary heart disease.

Materials and methods: Plasma lipoprotein subfractions were measured with the ion mobility method in 1575 subjects (45–74 years) from the Malmö Diet and Cancer cohort that during 12 years of follow-up were diagnosed with incident coronary heart disease and 1570 controls matched by sex and age.

Results: After adjusting for potential confounders, the highest versus lowest quintile of very small LDL (OR, 1.28; 95% CI, 1.02–1.61; P -trend, 0.008), small LDL (OR, 1.68; 95% CI, 1.33–2.12; P -trend, <0.001) medium LDL (OR, 1.54; 95% CI, 1.22–1.93; P -trend, <0.001), large IDL (OR, 1.26; 95% CI, 1.00–1.58; P -trend, 0.008) and VLDL (OR, 1.25; 95% CI, 1.00–1.58; P -trend, 0.006) was associated with higher risk of coronary heart disease, while no significant association was observed for large LDL (P -trend, 0.66) and small IDL (P -trend, 0.76). In addition, large HDL (OR, 0.57; 95 % CI, 0.44–0.73; P -trend, <0.001), but not small HDL (P -trend, 0.71), was associated with decreased risk.

Conclusion: In this prospective study measuring lipoprotein subfraction particle concentration in nonfasting blood, high concentrations of small and medium LDL particles and low concentration of large HDL particles showed the most predictive associations with coronary heart disease.

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Antibodies towards HDL components in type 2 diabetes patients are associated with modifications in the anti-atherogenic properties of HDL

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Background and aims: Type 2 diabetes is primarily a metabolic disorder, with a major vascular involvement. In patients with type 2 diabetes, the risk of developing atherosclerosis at an earlier age is three-to fivefold greater than in nondiabetics after controlling for other risk factors. Anti-atherogenic properties of HDL are well recognised: they prevent the oxidative modification of LDL and its consequent uptake by monocytes and inhibits cytokine-induced adhesion molecule production. We have previously identified anti-HDL (aHDL) antibodies in different medical conditions and associated them with changes in the anti-oxidant and anti-inflammatory properties of HDL. This study was undertaken to determine the presence of antibodies directed against different components of HDL in type 2 diabetes patients and establish a possible relationship between these antibodies and the anti-oxidant and anti-inflammatory properties of HDL.

Materials and methods: Thirty five type 2 diabetes patients were compared with an age and sex-matched control. IgG antibodies against HDL, apolipoproteins (Apo A-I, A-II and C-I) and paraoxonase 1 (PON1) were determined by ELISA, as was vascular and intercellular adhesion molecules (VCAM-1 and ICAM-1). Plasma lipid profile was determined by standard enzymatic techniques. PON 1 activity was assessed by quantification of nitrophenol formation. Nitric oxide metabolites (NOx) were measured by Griess reaction.

Results: Patients with type 2 diabetes had higher titres of IgG aHDL ($p<0.0001$), aApo A-I ($p=0.005$), and aPON ($p<0.0001$) antibodies, lower

PON1 activity ($p<0.0001$) and mean levels of HDL ($p=0.0003$) and increased levels of VCAM-1 ($p=0.037$), ICAM-1 ($p=0.018$) and NOx ($p=0.014$) than healthy controls. There was no difference in aApo A-II and aApo C-I antibodies. IgG aHDL antibodies directly correlated with aApo A-I ($p=0.022$), and aPON1 ($p=0.006$) levels. aApo A-I and aPON1 antibodies were associated with a decreased PON1 activity ($p=0.023$ and $p=0.004$) and increased endothelial dysfunction assessed by VCAM-1 ($p=0.005$ and $p=0.023$), ICAM-1 ($p=0.004$ and $p=0.002$) and NOx ($p=0.028$ and $p=0.007$).

Conclusion: In this study, IgG aHDL, aApo A-I and aPON1 antibodies were shown to be present in in type 2 diabetes, with no clinical features of autoimmune disease. These results suggest that aHDL antibodies might be a “family” of auto-antibodies of which Apo A-I and PON1 seem to be the main targets. These antibodies are associated with lower PON1 activity and higher VCAM-1, ICAM-1 and NOx and may contribute to the pathogenesis of atherosclerosis.

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Comparison of lipid variables to predict cardiovascular disease in Japanese hypercholesterolaemic patients with and without type 2 diabetes mellitus

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Background and aims: The several lipid variables and their ratios have been suggested to be predictive of CVD in patients with and without diabetes mellitus (DM), few studies have compared these variables directly in these patients, especially in Asia.

Materials and methods: We compared the relationship between CVD and various lipid variables (low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], non-HDL-C [NHDL-C], the ratio of LDL-C to HDL-C [LDL-C/HDL-C], the ratio of total cholesterol (TC) to HDL-C [TC/HDL-C]), and triglycerides (TG) in 3,170 hypercholesterolemic Japanese patients with and without DM in the diet alone group of the large-scale MEGA Study. DM was defined as a baseline fasting glucose ≥ 7.0 mmol/L, HbA1c $\geq 6.1\%$, or taking an oral hypoglycemic agent. DM ($n=2,502$) and non-DM ($n=668$) patients were divided by tertiles according to their baseline data for each lipid variable to compare the incidence of CVD, defined as coronary heart disease (CHD) plus stroke in the two groups. The hazard ratios (HR) and 95% confidence intervals (95% CI) were estimated by the Cox proportional hazard model.

Results: The Table summarizes the HR for CVD against the lowest risk tertile for each lipid parameter. The LDL-C/HDL-C and TC/HDL-C ratios were markedly and significantly higher in the highest tertile of non-DM (2.82 and 2.54, respectively) and in the middle tertile of DM patients (3.11 and 2.78, respectively). In non-DM patients, NHDL-C was associated with a significant 2.68 higher risk for CVD.

Conclusion: The best predictor of CVD in DM and non-DM Japanese hypercholesterolemic patients is the LDL-C/HDL-C and TC/HDL-C ratios.

Hazard ratios for CVD according to tertiles of various lipid variables (* $p<0.05$)

		LDL-C	HDL-C	TG	TC/HDL-C	LDL-C/ HDL-C	NHDL-C
Non DM	Tertile (low)	1	2.29*	1	1	1	1
	Tertile (middle)	1.31	1.59	1.10	1.94	2.01	1.82
	Tertile (high)	1.79	1	1.45	2.54*	2.82*	2.68*
DM	Tertile (low)	1	2.18	1	1	1	1
	Tertile (middle)	1.56	2.37*	1.13	2.78*	3.11*	0.97
	Tertile (high)	1.73	1	1.10	2.58*	2.87*	1.56

Clinical Trial Registration Number: NCT 00211705

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Greater dissociation of apolipoprotein B and LDL cholesterol targets in diabetes versus non-diabetes patients receiving lipid-lowering therapy

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Background and aims: Recent reports suggest apolipoprotein (apo) B is a good predictor of coronary risk. Low density lipoprotein cholesterol (LDL-C) may underestimate the number of atherogenic particles in certain patients with elevated levels of small dense LDL particles and remnant particles. To this end, the ADA/ACC treatment guidelines for cardiometabolic patients, including patients with diabetes, recommend apo B targets <0.9 g/L and <0.8 g/L for high risk and very high risk patients, respectively.

Materials and methods: This was a pooled analysis of 27 previously published, randomized, double-blind, active or placebo-controlled clinical trials conducted in 21794 adult patients (age range: 18–81 yr) with elevated LDL-C (range: 1.81–6.48 mmol/L) receiving ezetimibe (EZE)/statin or statin alone for 4 to 24 wk. Simple linear regression analyses were employed to calculate average LDL-C levels corresponding to apo B values of 0.9 g/L at baseline (i.e., in drug-naïve or statin-treated patients) and following treatment with EZE/statin or statin alone in subgroups of patients with/without hypertriglyceridemia (i.e., baseline TG $<$ and >2.26 mmol/L) and diabetes (either type 1 or 2).

Results: In diabetes and non-diabetes patients, LDL-C was highly correlated with apo B at baseline and correlations improved after treatment with EZE/statin and statin. At baseline in drug-naïve patients, the predicted LDL-C values, assuming an apo B=0.9 g/L, were close to the recommended treatment goals for high-risk patients (i.e., 2.59 mmol/L). At baseline in statin-treated patients and in both statin and drug-naïve patients following treatment with EZE/statin or statin alone, the predicted LDL-C values were closer to the more intensive recommended treatment goal (i.e., 1.81 mmol/L). In general, diabetes patients had lower predicted LDL-C values than non-diabetes patients irrespective of whether they were taking lipid-modifying drugs at baseline or not. When the linear regression analyses were examined by baseline TG, the predicted LDL-C values, assuming an apoB value of 0.9 g/L, were consistently lower in hypertriglyceridemic compared with normotriglyceridemic patients irrespective of diabetes status (data not shown).

Conclusion: Compared to non-diabetes patients, more aggressive LDL-C goals should be achieved in diabetes patients receiving lipid-lowering therapy to normalize apo B-containing lipoproteins.

Untreated at baseline or on treatment at endpoint	Population	N	Apo B:LDL-C Pearson Correlation Coefficient (R^2)	Predicted LDL-C corresponding to apo B=0.9 g/L
Drug naïve patients (i.e., patients washed off of lipid-modifying drugs at baseline)				
Baseline	Overall	12287	0.825 (0.681)	103.2 mg/dL
	Diabetes	3244	0.832 (0.692)	96.7 mg/dL
	Non-Diabetes	9043	0.806 (0.650)	108.0 mg/dL
EZE/statin	Overall	6109	0.903 (0.815)	78.4 mg/dL
	Diabetes	1526	0.883 (0.780)	74.5 mg/dL
	Non-Diabetes	4583	0.907 (0.823)	79.5 mg/dL
Statin Alone	Overall	6129	0.907 (0.823)	82.9 mg/dL
	Diabetes	1712	0.880 (0.774)	80.2 mg/dL
	Non-Diabetes	4417	0.912 (0.832)	84.0 mg/dL
Statin treated patients (i.e., patients treated with statin monotherapy at baseline)				
Baseline	Overall	9083	0.864 (0.746)	87.1 mg/dL
	Diabetes	3129	0.839 (0.704)	86.5 mg/dL
	Non-Diabetes	5954	0.865 (0.748)	88.4 mg/dL
EZE/statin	Overall	4773	0.875 (0.766)	78.3 mg/dL
	Diabetes	1813	0.875 (0.766)	75.4 mg/dL
	Non-Diabetes	2960	0.870 (0.757)	80.2 mg/dL
Statin Alone	Overall	3644	0.877 (0.769)	84.4 mg/dL
	Diabetes	1268	0.859 (0.738)	83.1 mg/dL
	Non-Diabetes	2376	0.883 (0.780)	85.1 mg/dL

apoB = apolipoprotein B; LDL-C= low-density lipoprotein cholesterol; EZE = ezetimibe 10 mg

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Anti-inflammatory and anti-oxidant properties of high density lipoproteins (HDL) are impaired in type 2 diabetesC. Morgantini^{1,2}, A. Natali³, B. Boldrini², S. Imaizumi³, M. Navab³, A. Fogelman³, E. Ferrannini², S. Reddy³;¹Scuola Superiore Sant'Anna, Pisa, ²Internal Medicine, University of Pisa, Italy, ³Medicine/Cardiology, University of California Los Angeles, USA.

Background and aims: Despite achieving recommended targets of serum cholesterol, blood pressure and glycaemia, type 2 diabetic (T2D) patients remain at high risk of vascular events; other factors must therefore be involved in this residual risk. Recent evidence suggests that the athero-protective role of HDL depends not only on their plasma concentration but also on their anti-inflammatory and anti-oxidant properties. In the present work, we sought to determine whether HDL function is impaired in T2D and whether *ex vivo* treatment with L-4F, an ApoA-I mimetic peptide, could improve HDL function.

Materials and methods: HDL was separated by FPLC from 93 T2D patients and 31 healthy subjects. The anti-inflammatory function of HDL was determined by measuring the ability of HDL to inhibit LDL-induced monocyte chemotactic activity in cultured human aortic endothelial cell monolayers. HDL inflammatory index (HII) was calculated as the monocyte migration induced by standard LDL (sLDL) in the presence of test HDL normalized for the value obtained with sLDL alone. The HDL anti-oxidant properties were measured by the cell-free assay (as the ratio between dichlorofluorescein diacetate fluorescence of HDL+sLDL vs sLDL alone) and also by HDL spontaneous fluorescence. In subgroups of patients and controls, HII was repeated after incubation with L-4F, and Liquid Chromatography-Mass Spectrometry measured the HDL content of 5 oxidized fatty acids.

Results: The mean HII was 1.42 ± 0.29 in diabetic subjects and 0.70 ± 0.19 in controls ($p < 0.001$). HDL anti-oxidant properties estimated either by the cell-free assay (2.03 ± 1.35 vs 1.60 ± 0.80 , $p < 0.05$) or with HDL spontaneous fluorescence (1708 ± 739 vs 1233 ± 601 rfu, $p < 0.001$) were impaired in T2D patients when compared with healthy subjects. HII correlated with spontaneous HDL fluorescence ($r = 0.23$) and with serum Lp(a) concentration ($r = 0.23$). *Ex-vivo* treatment of plasma with L-4F restored HDL anti-inflammatory activity in T2D (1.26 ± 0.17 vs 0.71 ± 0.11 after treatment, $p < 0.001$) and while only marginally improving it in healthy subjects (0.81 ± 0.16 vs 0.66 ± 0.10 after treatment, $p < 0.05$). Each and all the measured oxidized fatty acids in HDL were much higher (from 18 to 63 fold) in T2D patients than in controls.

Conclusion: In patients with T2D, the anti-inflammatory and anti-oxidant activities of HDL are severely impaired. L-4F, an ApoA-I mimetic peptide, *ex vivo* significantly improved HDL function; this novel approach might therefore result useful in the prevention of cardiovascular diseases in T2D.

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The effect of increased body fat accumulation on cardiometabolic risk factors in type 1 diabetes patients

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Background and aims: Increased body fat accumulation associated to increased prevalence of cardiometabolic risk factors is a frequent encounter and characterizes a "metabolically abnormal" phenotype of body fat increase in type 2 diabetes patients. Less data are available regarding the metabolic consequences of body fat accumulation in type 1 diabetes patients. The aim of this study was to evaluate the effects of increased fat accumulation in a group of type 1 diabetes patients.

Materials and methods: Clinical, biochemical and body composition data (using a bioimpedance analyzer) were compared across body fat mass (BFM) distribution tertiles in a group of one hundred and two (31 males, 71 females) consecutive type 1 diabetes patients. Data are presented as mean (SD) value. An EpiInfo software was used for statistical analysis. A p value < 0.05 was significant.

Results: There were no significant differences in BMI between males and females. As compared to the first tertile, the patients in the third tertile (T3) of BFM distribution had greater BMI [$29.5(2.9)$ vs $22.6(2.3)$ Kg/m²; $p < 0.0001$], BFM [$30.7(6.0)$ vs $14.1(3.9)$ Kg; $p < 0.0001$], estimated visceral (eVFA) fat area [$135.5(27.7)$ vs $79.3(22.1)$ cm²; $p < 0.0001$], waist circumference [$95.1(9.2)$ vs $80.0(7.3)$ cm; $p < 0.0001$] and disease duration [$16.9(9.6)$ vs $9.2(9.0)$ yrs;

$p < 0.002$] but no significant differences regarding age [$45.5(16.9)$ vs $43.7(14.7)$ yrs; $p = 0.63$], HbA1c [$9.0(1.9)\%$ vs $9.6(2.7)\%$; $p = 0.48$], serum uric acid [$3.5(1.5)$ vs $3.5(1.9)$ mg/dl; $p = 0.97$], plasma HDL-cholesterol [$53.0(15.0)$ vs $53.6(15.7)$ mg/dl; $p = 0.88$] and triglycerides [$98.4(50.2)$ vs $130.9(145.5)$ mg/dl; $p = 0.48$]. The prevalence of macrovascular complications (20.0% vs 18.2%; $p = 0.83$) was not different between groups. In logistic regression, macrovascular complications were significantly related to age but not to BFM or eVFA. The prevalence of hypertension (51.5% vs 32.1%; $p = 0.13$) and of microvascular complications (54.5% vs 31.8%; $p = 0.097$) were greater in T3 group but the differences did not reach statistical significance. In logistic regression, the prevalence of microvascular complications was related to disease duration but not to BFM or eVFA.

Conclusion: Our data suggest that increased weight and fat accumulation in type 1 diabetes patients is not associated with increased prevalence of cardiometabolic risk factors and macrovascular complications.

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Lipids seasonal variability in type 2 diabetes

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Background and aims: Previous studies conducted in healthy volunteers and in patients with coronary heart disease showed seasonal variation in lipid levels, with higher cholesterol levels in fall and winter than in spring and summer. No data were reported for diabetic patients. The aim of the study was to evaluate the seasonal variability of lipids in type 2 diabetic patients (DM2).

Materials and methods: In a retrospective study a group of 302 (183 F, 119 M) DM2 outpatients, aged 64.5 ± 9.0 yrs, were consecutively evaluated for two years during fall/winter (F/W) and spring/summer (S/S). All patients studied had unmodified lipid and hypoglycaemic drug therapies and, when present, the insulin doses were not changed by $> 10\%$ throughout the observation. In all patients, we collected anthropometrical indices, HbA1c, and lipid profile: total cholesterol (TC), high density lipoprotein (HDL-C), triglycerides (Trg), low-density lipoprotein (LDL-C) and non-HDL (non-HDL-C).

Results: The outpatients showed HbA1c $7.1 \pm 0.8\%$ with 6.6 ± 5.5 yrs duration of diabetes. 89.0% assumed metformin (Met), 7.4% thiazolidinediones, 30.2% sulphonylureas, 28.2% Met+SU in association and 11.4% were insulin treated. Among patients studied, 62.4% were currently treated with hypertensive drugs and 45% with statins. During the four periods of collection data, HbA1c levels showed seasonal variability with median 6.82% in F/W and 6.6% in S/S, without statistical significance. Instead, a statistically significant seasonal difference was observed for lipids, with the higher median levels in F/W than in S/S, both in patients without or under statin treatment. In particular, median TC levels in F/W were 196.5 mg/dl vs 189 mg/dl ($p < 0.001$) in subjects without statins and 167 mg/dl vs 165 mg/dl ($p < 0.05$) in patients with statins ($p < 0.05$). Median LDL-C levels observed were 120 mg/dl in F/W vs 111 mg/dl in S/S ($p < 0.001$) without statins and 88.7 mg/dl vs 86.5 mg/dl with statins ($p < 0.05$). A significant increase between F/W and S/S of HDL-C levels was observed only in males both without (42.5 mg/dl vs 44 mg/dl; $p < 0.001$) and with statin therapy (40 mg/dl vs 42 mg/dl; $p < 0.05$), respectively. Higher significant median Trg levels were observed in F/W versus S/S, showing 128.5 mg/dl vs 125 mg/dl ($p < 0.05$) for untreated patients and 126 mg/dl vs 123 mg/dl ($p < 0.05$) in patients with statins. Median non-HDL-C levels were also significantly elevated in F/W vs S/S: 145 mg/dl vs 137 mg/dl ($p < 0.001$) for untreated subjects and 114 mg/dl vs 110 mg/dl ($p < 0.001$) in patients with statins. When lipid targets were examined by seasons, rates of achievement were greater in S/S than F/W. In fact, the target of LDL-C < 100 mg/dl was reached by 27% patients in S/S vs 23.3% in F/W in the group without statins ($p < 0.05$) and by 70.7% vs 67% with statin therapy ($p < 0.05$). The target of non-HDL-C < 130 mg/dl was achieved by more subjects in S/S than in F/W only for patients with statins (51.6% vs 34%; $p < 0.001$). Moreover, Trg levels < 150 mg/dl were reached by more patients in S/S than in F/W: 75% vs 70% without lipid therapy, 73% vs 68% with statins and 66.7% vs 50% with other lipid drugs ($p < 0.001$, for all).

Conclusion: In DM2 clinical outpatients, for the first time, we observed a seasonal variability of lipids, with a peak in fall/winter and nadir in spring/summer. These data could suggest a less aggressive lipids treatment during the warmer seasons in order to increase the compliance of DM2 patients to the multitherapy.

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Low to moderate amounts of sugar sweetened beverages impair glucose and lipid metabolism, including beta-oxidation of fatty acids, in healthy young men - a randomised controlled trial

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Background and aims: Sugar-sweetened beverages (SSB) have unfavorable effects on glucose and lipid metabolism if consumed in high quantities by obese subjects but the effect of lower doses in normal weight subjects is less clear. The aim was to investigate the effects of SSB, consumed in small to moderate quantities for three weeks on LDL particle distribution, acylcarnitine profiles and other parameters of glucose and lipid metabolism in healthy young men.

Materials and methods: 29 subjects were studied in a prospective, randomized, controlled cross-over trial. Six three-week interventions were assigned in random order: (1-5) 600 ml SSB containing 40 or 80 g of fructose (medium/high fructose, MF/HF), or glucose per day (medium/high glucose, MG/HG) or 80 g of sucrose per day (high sucrose, HS); (6) dietary advice to consume low amounts of fructose (LF). Outcome parameters were measured at baseline and after each intervention. In addition, fasting acylcarnitine profiles were measured by mass spectrometry in a second group of 9 subjects undergoing the same interventions.

Results: LDL-particle size was reduced after HF by -0.51nm (95% CI -0.19 to -0.82nm) and after HS by -0.43nm (-0.12 to -0.74 , $p<0.05$ for both). Similarly, a more atherogenic LDL subclass distribution was seen when fructose containing SSB were consumed (MF, HF and HS, $p<0.05$). Fasting glucose increased significantly after all interventions (4-9%, $p<0.05$). Levels of long- and also medium-chain fasting acylcarnitines were increased after fructose and sucrose diets (HF, MF and HS), compared to glucose (HG) diets (e.g. C18 acylcarnitines HF $2.38\text{ }\mu\text{mol/l}$ vs HG 1.81 ; C10-C18 acylcarnitines HF $4.95\text{ }\mu\text{mol/l}$ vs HG 3.62 , C16 (palmitoylcarnitine) HF $1.43\text{ }\mu\text{mol/l}$ vs HG 0.96 ; all $p<0.05$).

Conclusion: The present data clearly demonstrate potentially harmful effects of low to moderate consumption of SSB on markers of cardiovascular risk such as LDL particles and fasting glucose. Analysis of acylcarnitine profiles is performed for the first time in this setting and yields further insights into fatty acid metabolism, suggesting impaired beta-oxidation of fatty acids after fructose containing diets.

Supported by: Swiss National Science Foundation

the group of T2D patients, plasma rbp4 was correlated positively with triglycerides ($r=0.74$, $p=0.003$), apoB VLDL pool ($r=0.62$, $p=0.017$) and negatively with VLDL-apoB FCR ($r=-0.66$, $p=0.001$) whereas these association were not found in the group of normal subjects. In the diabetic population rbp4 level explained 44% of VLDL-apoB FCR variance and the strong negative association between plasma rbp4 and VLDL-apoB FCR was independent of age, gender, glycemia or BMI.

Conclusion: 1) Our kinetic study has shown, in T2D patients, but not in normal subjects, a strong negative correlation between plasma rbp4 and VLDL-apoB FCR. This can explain the positive correlation between plasma rbp4 and triglycerides, in T2DM. 2) These data suggest that rbp4 may be involved in the pathophysiology of hypertriglyceridemia in T2DM by reducing VLDL catabolism.

Clinical Trial Registration Number: ClinicalTrials.gov NCT00658463

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Retinol binding protein 4 (rbp4) is a significant determinant of VLDL catabolism in patients with type 2 diabetes

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Background and aims: Plasma levels of the adipokine retinol binding protein 4 (rbp4), that are elevated in patients with type 2 diabetes, have been shown to be positively correlated with triglycerides. However, so far, the association between rbp4 and triglyceride metabolism remains unknown. This prompted us to perform a study in order to analyse the association between plasma rbp4 and apoB-containing lipoproteins.

Materials and methods: An *in vivo* kinetic study with stable isotopes (¹³C-leucine) was performed in 34 subjects including 14 patients with type 2 diabetes (T2D) and 20 normal individuals. Plasma rbp4 was measured by ELISA.

Results: Plasma rbp4 levels were significantly higher in T2D patients than in normal subjects (41.6 ± 15.9 vs. $31.3 \pm 10.1\text{ }\mu\text{g/ml}$, $p<0.05$). In the whole studied population, plasma rbp4 was correlated positively with triglycerides ($r=0.61$, $p<0.001$), apoB VLDL pool ($r=0.60$, $p<0.001$) and negatively with VLDL-apoB Fractional Catabolic Rate (FCR) ($r=-0.39$, $p=0.025$). No association was found between plasma rbp4, on the one hand, and VLDL-apoB production rate, apoB-IDL or LDL kinetic parameters, on the other hand. In

PS 050 Lipoprotein metabolism

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Central role of lipoprotein lipase and very low-density-lipoprotein receptor on macrophage foam cell formation by triglyceride-rich lipoproteins and low density lipoprotein

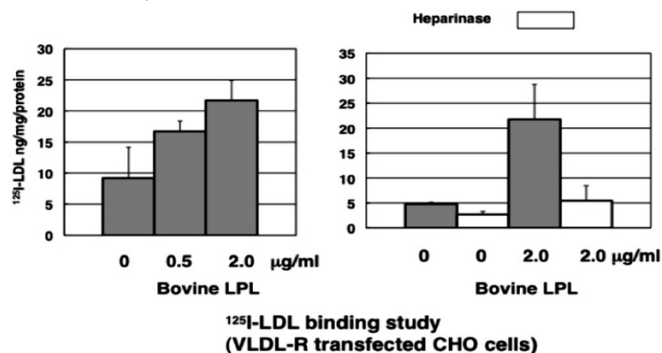
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Background and aims: Dyslipidemia is a common feature of diabetes and is related to cardiovascular disease. Triglyceride-rich lipoproteins (TGRLs) and low-density-lipoprotein (LDL) cholesterol are independent risk factors for coronary artery disease. VLDL receptor (VLDL-R) is a member of the low-density-lipoprotein receptor (LDL-R) family. VLDL-R binds and internalizes TGRLs with high specificity and is not down regulated by sterols. VLDL-R was expressed abundantly in fatty acids active tissues (heart, skeletal muscle and fat), brain and macrophages. In macrophages, VLDL-R is one of the candidate receptors for the foam cell formation by TGRLs, and macrophage cells itself secrete apolipoprotein (apo) E and lipoprotein lipase (LPL). We have reported that the low affinity binding of fasted human VLDL to the VLDL-R could be overcome by enriching VLDL with either apoE or LPL. In this study, we investigated the role of apoE, LPL and VLDL-R on foam cell formation in macrophages.

Materials and methods: We used human THP-1 macrophages and human monocyte-derived macrophages because there is no VLDL-R protein in mouse macrophages. Northern- and Western-blot were used to examine the expression of apoE, LPL and VLDL-R. LPL protein levels in medium were also measured. To check the foam cell formation by TGRLs in macrophages, oil-red stain was used. 125 I-lipoprotein binding study was also examined.

Results: Beta-VLDL (TGRLs) and oxidized LDL but not native LDL induced foam cell formation in macrophages. During macrophage differentiation, the expression of VLDL-R, LPL, apoE and type A scavenger receptor made an appearance, and the expression of LDL-R was decreased. Interferon-gamma (IFN- γ) inhibited the VLDL-R and LPL expression in a dose- and time-dependent manner. The apoE and LDL-R expression were not changed by IFN- γ . IFN- γ inhibited the foam cell formation by TGRLs in parallel. Furthermore small interfering (Si) RNA of VLDL-R or LPL and anti-LPL monoclonal antibody (5D2) decreased the foam cell formation by TGRLs. Using VLDL-R over-expressing IdLA-7 CHO cells (LDL-R deficient CHO cells), we showed that native LDL that is not recognized by VLDL-R was taken up by the VLDL-R when native LDL was incubated with LPL. The effect of LPL on the up-take of LDL by the VLDL-R was decreased by heparinase treatment (graph).

Conclusion: It is likely that VLDL-R is functioning in concert with LPL in macrophages for the uptake of both TGRLs and native LDL. Surprisingly native LDL particles are recognized by VLDL-R when LDL is modified by LPL. Inhibition of LPL or VLDL-R expression may attenuate an early atherosclerosis in diabetes by TGRLs and LDL.



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Plasma phospholipid transfer protein activity is independently determined by obesity and insulin resistance

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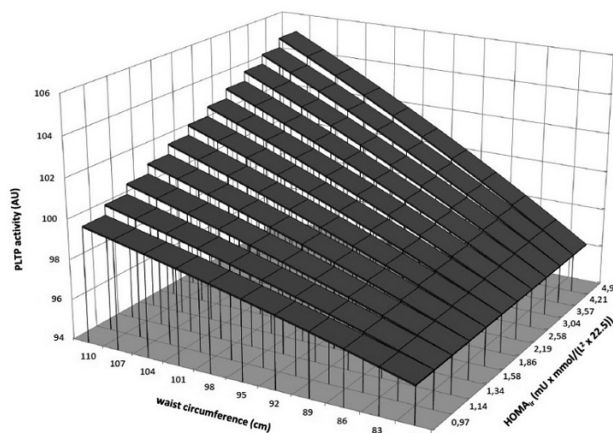
Background and aims: Phospholipid transfer protein (PLTP) is an emerging cardio-metabolic risk factor which is intricately involved in lipoprotein metabolism. Elevated plasma PLTP activity levels have been reported in diabetes mellitus and obesity, but the relative contributions of obesity and insulin resistance to plasma PLTP activity remain unclear. We tested whether plasma PLTP activity is independently related to (central) obesity and insulin resistance in non-diabetic subjects.

Materials and methods: The relationships of plasma PLTP activity levels (phospholipid vesicles-HDL system) with waist circumference, waist/hip ratio, BMI and insulin resistance (homeostasis model assessment (HOMA_{ir})) were determined in 313 non-diabetic subjects (273 men).

Results: PLTP activity was higher in 67 subjects with enlarged waist circumference (NCEP-ATP-III criteria; 102±11 AU) compared to 246 subjects with normal waist (98±11 AU, P=0.027). In univariate analysis PLTP activity correlated positively with BMI (r=0.125), waist (r=0.188), waist/hip ratio (r=0.143) and HOMA_{ir} (r=0.192) (p<0.05 to p<0.001). In age- and sex-adjusted multiple linear regression models, waist circumference (β=0.158, p=0.025) but not BMI (P=0.55) predicted PLTP activity independently of HOMA_{ir} (β=0.126, p=0.047). Additionally, both waist circumference and waist/hip ratio interacted positively with HOMA_{ir} on PLTP activity (β=0.109, p=0.056 and β=0.156, p=0.034, respectively). The interaction of waist circumference with HOMA_{ir} on PLTP activity is shown in the Figure.

Conclusion: In non-diabetic subjects, both (central) obesity and insulin resistance influence plasma PLTP activity, resulting in further elevated plasma PLTP activity with increasing obesity and insulin resistance. Higher PLTP activity could contribute to elevated cardiovascular risk attributable to obesity and insulin resistance.

Figure: Graphical presentation of the interaction between waist circumference and insulin resistance on plasma PLTP activity



Supported by: Dutch Diabetes Research Foundation

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Cholesterol-lowering effects of metformin in APOE*3-Leiden.CETP mice, a transgenic model with a human-like lipoprotein profile

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Background and aims: Metformin is one of the most widely used antidiabetic drugs in the treatment of type 2 diabetes. On top of its anti-hyperglycemic properties, metformin also lowers plasma very low-density lipoprotein

(VLDL) in type 2 diabetes patients by a mechanism that remains to be elucidated. The aim of this study was to investigate the effects and underlying molecular mechanisms of metformin on VLDL metabolism in APOE*3-Leiden.CETP mice, a transgenic model characterized by a human-like lipoprotein profile.

Materials and methods: 3 months-old APOE*3-Leiden.CETP female mice were fed a Western-type diet containing 0.1% cholesterol for 4 weeks, followed by the same diet with or without metformin (250 mg/kg body weight/day) for 4 weeks. Body weight, food intake, plasma glucose, insulin, total cholesterol (TC), triglycerides (TG), phospholipids (PL) and free fatty acids (FFA) levels, and plasma lipoproteins profile were measured at week 0, 2 and 4. Hepatic VLDL-TG production and tissue-specific clearance of VLDL-like emulsion particles were determined at week 4.

Results: Metformin did not affect body weight, food intake and plasma glucose, insulin and FFA levels but significantly lowered plasma TC (-30% and -38% at week 2 and 4, respectively; $p < 0.05$) and TG (-28% and -29% at week 2 and 4, respectively; $p < 0.05$). The analysis of lipoproteins profile revealed that the cholesterol-lowering effects of metformin is mainly due to a decrease in VLDL (-37%) without obvious change in high-density lipoprotein (HDL) levels. Metformin did not affect hepatic VLDL-TG production rate and particle composition, suggesting that the drug might rather promote VLDL-TG clearance by peripheral tissues. Interestingly, metformin specifically increased VLDL-derived FA retention in brown adipose tissue after continuous infusion of radiolabeled VLDL-like emulsion particles (+58%; $p < 0.05$).

Conclusion: Metformin decreases plasma VLDL-TG levels in APOE*3-Leiden.CETP mice by a mechanism which does not involve change in hepatic VLDL production but apparently promotes VLDL clearance by brown adipose tissue.

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APOE*3Leiden.CETP transgenic mice as a model for the metabolic syndrome

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Background and aims: The metabolic syndrome is characterized by the co-occurrence of several risk factors i.e. increased body weight (bw) and insulin resistance (IR) and at the same time adverse changes in plasma lipids as observed in diabetic dyslipidemia, with increased triglycerides and apoB-containing lipoproteins and decreased HDL. The aim was to investigate whether the APOE*3Leiden.CETP (E3L.CETP) mouse is a translational model for the metabolic syndrome.

Materials and methods: Male E3L.CETP mice were put on a high fat diet and fructose in drinking water for 12-16 weeks to induce diet-induced obesity and IR. Thereafter, the mice were treated with either rosiglitazone (3 and 11 mg/kg/d), liraglutide (0.2 mg/kg/d), HSD-1 inhibitor (0.1 mg/kg/d), resveratrol (75 mg/kg/d), fenofibrate (12 mg/kg/d), atorvastatin (10 mg/kg/d) or niacin (720 mg/kg/d) for 4 weeks. The effects on bw, plasma lipids and IR (via plasma glucose/insulin and/or hyperinsulinemic euglycemic clamps) were assessed.

Results: Dietary treatment resulted in a human-like lipoprotein profile with a TC/HDL-C ratio of 3-4. Anti-diabetic compounds rosiglitazone, liraglutide and HSD-1 inhibitor significantly decreased glucose and insulin levels or IR. Liraglutide and HSD-1 inhibitor also decreased bw. Established lipid-lowering compounds atorvastatin, fenofibrate and niacin, and resveratrol improved the dyslipidemia.

Conclusion: The data indicate that the E3L.CETP mouse is a good translational animal model combining all important features that underlie the metabolic syndrome and mimic the human response to clinically used treatments. We conclude that the E3L.CETP mouse is a promising model to investigate the effects of new drugs, alone or in combination, that affect IR and diabetic dyslipidemia.

PS 051 Hepatic lipid signalling

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Kupffer cells as mediators of the beneficial metabolic effects of thiazolidinediones

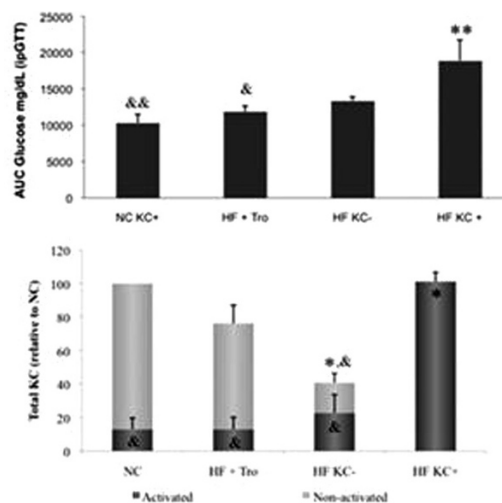
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Background and aims: Kupffer cells (KC), the liver resident macrophages, have been implicated in the pathogenesis of the hepatic inflammation, insulin resistance, and steatosis associated with obesity/T2DM. Thus, high fat feeding rapidly induces a M1 inflammatory profile in KC, and KC depletion is sufficient to prevent the hepatic insulin resistance and steatosis associated with high fat feeding. Of interest, thiazolidinediones (TZD) improve hepatic insulin sensitivity, but the mechanisms of action remain unclear. Here, we tested the hypothesis that the effects of TZD are associated with a suppression of high fat feeding induced M1 KC polarization.

Materials and methods: Male Wistar rats (200-220 g) were submitted to surgery for implantation of chronic indwelling catheters in the left common carotid artery. After recovery, animals were separated in 4 different study groups: Group 1. NC: Rats maintained on a normal chow diet (n=5); Group 2. HF/KC+: Rats maintained on a high fat custom diet (Tekland TD.96001, Harlan Laboratories, Madison, WI, USA) (n=3); Group 3. HF/Tro: HF diet-fed rats treated with 0.02% of the TZD Troglitazone (n=4); and finally Group 4. HF/KC-: HF-fed rats KC depleted by means of 3-4 day intra-carotid interval injection of 10 mg/kg bw Gadolinium (III) chloride (Sigma, St. Louis, MI, USA). Following 14 days of diet, all animals underwent an i.p. glucose tolerance test (2 mg glucose/g bw). KC depletion was evaluated by qRT-PCR quantification of F4/80 mRNA levels and KC polarization in liver samples was measured by macrophage-localized TNF- α Immunofluorescence detection.

Results: Our data demonstrate that HF/KC+ had reduced glucose tolerance and increased KC polarization compared to NC. High fat fed KC-depleted animals (HF/KC-) displayed improved glucose tolerance concomitant with decreased KC number. Finally, TZD treatment ameliorated glucose intolerance in HF rats and these effects were associated with markedly reduced KC M1 polarization (See Figure).

Conclusion: Our data indicate that KC are important targets and potential mediators of the hepatic insulin-sensitizing actions of TZD in the liver. Further studies involving the metabolic profiling of KC-depleted and TZD-treated overfed rats are necessary to validate our hypothesis.



Area under the curve (AUC) during an intraperitoneal glucose tolerance test (2.0 mg glucose/g bw) and percentage of activated KC (measured as percentage of TNF- α releasing macrophages detected by Immunofluorescence Microscopy) and total KC (measured as gene expression of F4/80, a transmembrane glycoprotein expressed in macrophages). Normal chow-fed rats correspond to NC (n=5). HF-fed rats treated with 0.02% Troglitazone correspond to HF + Tro (n=4). HF-fed KC-depleted rats correspond to HF KC- (n=5) and HF-fed rats correspond to HF KC+ (n=3). * $p < 0.05$, relative to NC; ** $p < 0.01$, relative to NC; Δ $p < 0.05$, relative to HF KC+ and $\Delta\Delta$ $p < 0.01$, relative to HF KC+.

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Prep1 regulates hepatic lipogenesis through SHIP2-mediated AMPK inhibitionS. Cabaro¹, F. Oriente¹, S. Iovino¹, V. D'Esposito¹, A. Liotti^{1,2}, F. Blasi³, C. Miele¹, P. Formisano¹, F. Beguinot¹;¹DBPCM & IEOS-CNR, University of Naples Federico II, ²Department of Pharmaceutical Science, University of Salerno, ³IFOM (Firc Institute of Molecular Oncology), Milan, Italy.

Background and aims: Prep1 is a homeodomain transcription factor belonging to the TALE proteins, including also Pbx1, which plays an essential role in hematopoiesis, organogenesis and development. Prep1 forms transcriptionally active complexes with Pbx1 and regulates the activity of several genes. The Prep1 null mutation leads to embryonic death at a very early stage. However, Prep1 heterozygous (Prep1^{i/+}) mice, which express only 55–57% of protein, have a complex metabolic phenotype with at least two relevant features. One is the presence of smaller but otherwise normally structured islets with reduced fasting and post-loading plasma insulin levels. The second is increased insulin sensitivity in skeletal muscle and in liver which is accompanied by protection from streptozotocin-induced diabetes. In muscle decreased Prep1 levels are followed by an increase of the PGC1α/ Glut4 mediated glucose uptake. In liver, better insulin sensitivity is due to a reduced Shp1 tyrosine phosphatase expression followed by an increase of insulin signaling. Also triglyceride levels are significantly reduced in the liver of Prep1^{i/+} mice. However, the molecular mechanism by which Prep1 controls lipogenesis is unclear. In this study we have focused our attention on the role of Prep1 on the regulation of the triglyceride synthesis.

Methods and results: To study the lipogenesis in the liver of the Prep1 heterozygous mice, we have examined the expression of the lipogenic enzyme FAS (Fatty Acid Synthase) by real-time RT-PCR analysis. Hepatic expression of FAS is significantly decreased. Western Blot analysis have shown increased phosphorylation of PKCζeta, LKB1, AMPK and ACC, which may control FAS expression and triglyceride production in Prep1^{i/+} mice liver. mRNA and protein levels of the lipid phosphatase SHIP2, an inhibitor of PI3Kinase/ PKCζeta signaling, are reduced by 40% in the liver of Prep1^{i/+} mice. Consistent with these data, HepG2 (Human Hepatocarcinoma cell line) cells overexpressing Prep1 show increased triglyceride levels and FAS expression, while PKCζeta, LKB1, AMPK and ACC phosphorylation is strongly reduced. Moreover, SHIP2 levels are increased by 50%. Interestingly, overexpression of Pbx1 cDNA in HepG2 cells mimicked Prep1 triglyceride synthesis. At the opposite, Prep1^{HR1} mutant, which is unable to bind Pbx1, fails to elicit these effects. Chromatin IP experiments indicate that Prep1/Pbx1 complex can bind SHIP2 promoter region and regulate its expression.

Conclusion: These data suggest that Prep1/Pbx1 regulates hepatic triglyceride production by increasing SHIP2 levels and thereby inhibiting the PKCζeta/AMPK signaling.

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Cross-talk between proteasome and autophagy plays a role in hepatic insulin resistance via endoplasmic reticulum stressT. Otoda¹, T. Takamura¹, H. Misu¹, H. Hayashi¹, T. Ota¹, M. Yamamoto², S. Iseki², S. Kaneko¹;¹Disease Control and Homeostasis, ²Histology and Embryology, Kanazawa University Graduate School of Medical Science, Japan.

Background and aims: Insulin resistance is a key feature of people with type 2 diabetes (T2D). Growing evidence has suggested that accumulation of endoplasmic reticulum (ER) stress in the liver is a major contributor to insulin resistance; however, the molecular mechanisms linking diabetes and ER stress are not fully understood. In the last meeting (46th EASD Annual Meeting), we reported that proteasome dysfunction is observed in the liver of a mouse model of diet-induced obesity and causes hepatic insulin resistance, at least partly, via ER stress. In that experiment, autophagy was induced in the liver of mice with impaired proteasome function. In the present study, we investigated the relationship between proteasome function and autophagy in the development of insulin resistance in a cultured hepatocyte cell line.

Materials and methods: We isolated liver from mice fed a high-fat diet for 28 weeks. Proteasome activity was measured by the chymotrypsin-like protease activity. Rat hepatoma-derived H4IIEC3 cells were incubated with bortezomib (BZ), a selective inhibitor of the 26S proteasome. Insulin signaling pathways were evaluated by western blotting.

Results: Insulin receptor (IR) protein, but not IRS-2 protein, was decreased by ~30% without changing IR mRNA levels in the livers of mice fed a high fat diet (p<0.05, n=4). Unexpectedly, in the liver of these mice, proteasome activity was rather inhibited by 30% (p<0.05, n=4). Based on these *in vivo* findings, we addressed whether and how proteasome dysfunction is sufficient to cause insulin resistance *in vitro* by using H4IIEC3 hepatocytes. A proteasome inhibitor BZ concentration-dependently (10 to 100 μmol/l) increased proteins involved in an unfolded protein response, such as BiP and CHOP, and phosphorylation of IRE1α and c-Jun NH2-terminal kinase (JNK). IR protein and insulin-stimulated serine phosphorylation of Akt were markedly reduced by 20 and 40%, whereas IRS-2 protein levels were rather increased by 20 % in 100 μmol/l BZ-treated H4IIEC3 hepatocytes. Because BZ decreased IR protein, we evaluated the non-proteasomal pathways of protein degradation. BZ elicited accumulation of LC3B-II, a lipidated form of LC3B localized on the autophagic vacuoles (AVs), in H4IIEC3 hepatocytes. Most convincingly, electron microscopic examination of BZ-treated cells indicated a considerable increase of AVs.

Conclusion: Autophagy is likely activated in response to ER stress caused by accumulated misfolded proteins during proteasome inhibition. Present data also suggest that degradation of IR is induced through the lysosomal pathway, whereas IRS-2 is degraded through the proteasomal pathway in hepatocytes. Collectively, we propose a previously unrecognized role of ubiquitin-proteasome and autophagy-lysosome system for the development of insulin resistance in the liver.

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Toll-like receptor-4 mediates obesity-induced nonalcoholic steatohepatitis through activation of x-box binding protein-1 in miceK.S.L. Lam^{1,2}, D. Ye¹, F.Y.L. Li¹, Y. Wang^{2,3}, K. Man⁴, C.M. Lo⁴, A. Xu^{1,2};¹Department of Medicine, ²Research Centre of Heart, Brain, Hormone and Healthy Aging, ³Department of Pharmacology & Pharmacy, ⁴Department of Surgery, The University of Hong Kong, China.

Background and aims: Nonalcoholic fatty liver disease (NAFLD) is an obesity-related chronic liver disorder ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which may progress to liver fibrosis and cirrhosis. This study aims to investigate the role of Toll-like receptor (TLR) 4 in mediating the transition from steatosis to inflammation.

Materials and methods: We generated ApoE^{-/-}/TLR4^{-/-} mice and ApoE^{-/-}/TLR4 wild type mice (ApoE^{-/-}/TLR4-WT) by cross-breeding the ApoE deficient (ApoE^{-/-}) strain with TLR4-deficient mice, which was then fed a high fat high cholesterol (HFHC) diet for 12 weeks to induce obesity.

Results: ApoE^{-/-}/TLR4-WT mice fed with high fat high cholesterol (HFHC) diet for 12 weeks developed typical pathological features of NASH in the context of obesity and metabolic syndrome. By contrast, ApoE^{-/-}/TLR4^{-/-} mice, which lack functional TLR4, were resistant to HFHC diet-induced liver inflammation and injury, and were less susceptible to the diet-induced production of reactive oxygen species (ROS) and proinflammatory cytokines. In ApoE^{-/-}/TLR4-WT mice, X-box binding protein-1 (XBP-1), a transcription factor involved in the unfolded protein responses, was activated in liver upon feeding with HFHC diet, whereas such an activation of XBP-1 was abrogated in ApoE^{-/-}/TLR4^{-/-} mice. In primary rat Kupffer cells, endotoxin induced XBP-1 activation through ROS production, whereas siRNA-mediated knockdown of XBP-1 expression resulted in a marked attenuation in endotoxin-evoked NF-κB activation and cytokine production. Furthermore, adenovirus-mediated expression of dominant negative XBP-1 in the liver led to a significant attenuation in HFHC diet-induced liver inflammation and injury in ApoE^{-/-} mice, but had little effect on hepatic lipid accumulation and ROS production.

Conclusion: These findings support the role of TLR4 in Kupffer cells as a key player in mediating the progression of simple steatosis to NASH, by inducing ROS-dependent activation of XBP-1.

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Feeding a vitamin C and E enriched diet impairs the normal metabolic response to physical exercise in the liver of mice

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Background and aims: The increased energy demand associated with prolonged physical exercise causes substantial changes in metabolite fluxes and

directly affects the liver, the key regulator of energy homeostasis. We could previously show that endurance exercise provokes a strong and acute response not only of metabolic, but also of stress-sensitive signalling pathways in the liver. Antioxidant intake with the aim of reducing oxidative stress has a high prevalence in the general population and among endurance athletes. However, excess intake of vitamin C and E could impair the adaptation of the muscle to physical exercise as well as beneficial effects on whole-body insulin sensitivity. We hypothesized that the response of the liver could be affected as well by antioxidant intake and attempted to clarify whether the underlying mechanisms are related to anti-oxidative or direct metabolic effects of these vitamins.

Materials and methods: Male C57BL/6 mice were supplemented during 4 weeks with control diet (n=12) or a vitamin C (100 mg/kg, control diet vitamin C-depleted) and vitamin E (2000 U/kg, control 149 U/kg) enriched diet (n=12) before 6 mice of each group were subjected to 1 h of treadmill running at non-exhaustive conditions (14 m/min, 14° uphill slope) while the others remained sedentary. Plasma and tissue metabolic parameters and mRNA levels in liver and skeletal muscle were assessed immediately after the bout of exercise.

Results: Plasma vitamin E concentration was two-fold increased after the diet (12.6 ± 0.6 vs. 6.5 ± 0.5 $\mu\text{mol/l}$, $p < 0.05$), and exercise-induced upregulation of the endogenous anti-oxidant metallothionein 1 (Mt1) in the liver was blunted in these mice. In contrast, the immediate early response of MAPK- and p53-dependent genes in the liver was not reduced by the vitamin C/E-diet. Most strikingly, the mice fed the vitamin C/E-diet did not show increased plasma free fatty acids after the run (784 ± 174 vs. 620 ± 139 $\mu\text{mol/l}$, $p < 0.05$). Well in line, induction of the PPAR α/δ target gene angiopoietin-like 4 (Angptl4) was prevented in liver and tibialis anterior muscle after exercise, as well as the hepatic increase in mRNA levels of the metabolic regulators PGC-1 α (Ppargc1a), pyruvate dehydrogenase kinase 4 (Pdk4) and insulin receptor substrate 2 (Irs2). The gluconeogenic enzyme G6Pase (G6p) was similarly induced in both groups, and liver glycogen content was not different after the run, while only mice fed the control diet had reduced plasma glucose levels afterwards (5.70 ± 0.50 vs. 7.24 ± 0.53 mmol/l in control, $p < 0.05$; and 7.09 ± 0.66 vs. 7.02 ± 0.29 mmol/l in vitamin C/E-fed mice). The trend towards lower triglyceride content in skeletal muscle after the run in vitamin C/E-fed mice further supports alterations predominantly in fatty acid metabolism.

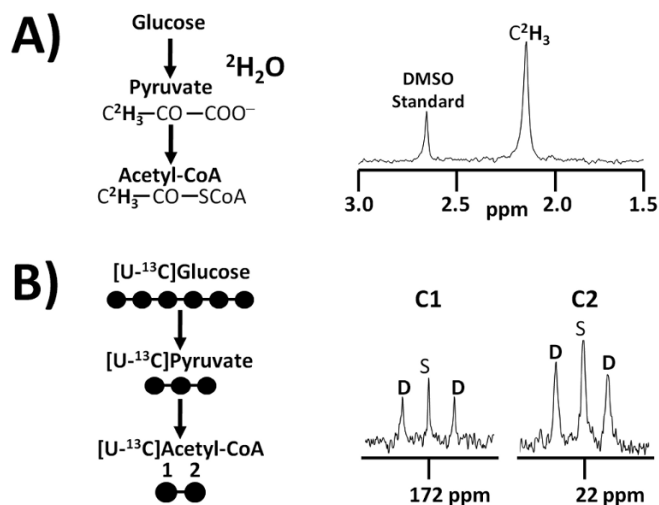
Conclusion: This study provides first evidence that the combination of vitamin C and E impairs the acute adaptive response to endurance exercise not only in the muscle, but also in the liver. The data suggest that this impairment could be due to metabolic rather than anti-oxidative effects. Possibly, vitamin E, which is closely linked to lipid turnover in the body, could directly interfere with fatty acid handling and thus affect the hepatic response to physical exercise.

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N-acetyl PABA was purified by solid phase extraction and the acetyl carbon and hydrogen enrichments from $2\text{H}_2\text{O}$ and $[\text{U-}^{13}\text{C}]\text{glucose}$ were determined by NMR.

Results: Fig 1A and B shows representative 2H and ^{13}C spectra of N-acetyl-PABA recovered from protocols 1 and 2, respectively. From protocol 1, enrichment of the acetyl CoA methyl hydrogens from $2\text{H}_2\text{O}$ was estimated to be 2.2 ± 0.3 % compared to 2.4 ± 0.3 % for body water. This indicates that hepatic acetyl-CoA and body water enrichments were equivalent under these conditions. In Figure 1B, the ^{13}C acetyl signals of the N-acetyl PABA is shown. For each carbon, the ^{13}C - ^{13}C -coupled doublets (D), originating from the $[\text{U-}^{13}\text{C}]\text{glucose}$ precursor are well resolved from the background natural abundance ^{13}C singlet (S) allowing the excess acetyl-CoA ^{13}C -enrichment from $[\text{U-}^{13}\text{C}]\text{glucose}$ to be calculated. This was estimated to be $1.7 \pm 0.1\%$.

Conclusion: Analysis of murine hepatic acetyl CoA enrichment from $[\text{U-}^{13}\text{C}]\text{glucose}$ and $2\text{H}_2\text{O}$ by PABA administration and NMR analysis of N-acetyl-PABA was demonstrated. The analyses revealed that a surprisingly small fraction of hepatic acetyl-CoA ($< 2\%$) was derived from an intraperitoneal glucose load given to 24-hour fasted mice. This suggests that the liver was not committed to glycolytic and lipogenic utilization of the administered glucose under these conditions. Following $2\text{H}_2\text{O}$ administration, hepatic acetyl-CoA and body water hydrogens are enriched to the same levels hence body water enrichment can be used as a surrogate for the true acetyl-CoA precursor.



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Noninvasive sampling of murine hepatic acetyl-CoA enrichment with p-amino benzoic acid

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Background and aims: Acetyl-CoA is a key hepatic metabolite but is difficult to analyze for enrichment from metabolic tracers. We developed a method that allows acetyl-CoA enrichment from ^{13}C - and 2H -enriched precursors to be quantified. p-Amino benzoic acid (PABA) is acetylated via hepatic acetyl-CoA and N-acetyl PABA is cleared into urine where it is analyzed. To demonstrate this sampling method, acetyl-CoA enrichment from deuterated water ($2\text{H}_2\text{O}$) was quantified by 2H NMR of N-acetyl-PABA in order to determine the true 2H -precursor enrichment for de novo lipogenesis. Second, the contribution of an $[\text{U-}^{13}\text{C}]\text{glucose}$ load to hepatic acetyl-CoA was determined by ^{13}C NMR analysis.

Materials and methods: In protocol 1, 4 mice were injected with $2\text{H}_2\text{O}$ in saline (3g/kg body water) one hour into the light phase, and their drinking water was also enriched with 3% $2\text{H}_2\text{O}$. 24 hours later, they received an injection of PABA (15 mg/kg) dissolved in saline containing 3% $2\text{H}_2\text{O}$. Urine was collected for 6 hours following PABA injection. In protocol 2, 4 mice were fasted overnight, then injected with $[\text{U-}^{13}\text{C}]\text{glucose}$ (2 g/kg) and PABA (15mg/kg) in saline. Urine was collected over the following 6 hours. Urinary

PS 052 Hepatic lipid metabolism

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Effects of acute exercise on hepatic lipid content

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Background and aims: There is increasing evidence that hepatic lipid content (IntraHepatic Lipid, IHL) markedly increases the risk of metabolic complications, including insulin resistance and cardiovascular events. Recent evidence suggests that exercise training might have beneficial effects on IHL content. However, it is unknown if an acute bout of exercise is able to lower IHL content. One confounding factor when investigating this research question is that acute exercise raises plasma fatty acid (FA) concentrations. We have previously shown that elevated concentrations of plasma FA, induced by exercise in the fasted state, lead to increased lipid accumulation in the heart and in non-active skeletal muscle. To circumvent this, we investigated the effect of acute exercise on IHL in both, the fasted and the glucose-fed state, as the increase in plasma FA concentrations is severely blunted in the latter condition. We hypothesized that acute exercise would be able to lower IHL content.

Materials and methods: Seven overweight male subjects (age: 57.4 ± 8.3 y, BMI: 28.4 ± 1.8 kg/m²) underwent hepatic Proton Magnetic Resonance Spectroscopy (¹H-MRS) (Intera, 1.5T, Philips Healthcare) in the morning in the fasted state to determine IHL content. Subsequently, subjects cycled for two hours at 50% of maximal performance. IHL content was measured directly after exercise and again four hours post-exercise. All subjects underwent this procedure twice, once while fasted, and once while ingesting glucose during both exercise and recovery from exercise (bolus: 1.4g/kg, 8 x 0.35 g/kg). Indirect calorimetry was used to determine whole body fat- and carbohydrate oxidation. Blood plasma samples were collected repeatedly for later analysis. For hepatic ¹H-MRS, an 18 cm³ VOI was placed within the lower right hepatic lobe (PRESS, TR = 4s, TE = 23ms, n = 64). Water signal was suppressed using frequency-selective prepulses. Spectra without water suppression were acquired with identical settings (n=64) and all spectra were fitted with AMARES in the jMRUI software. Values are given as T2-corrected ratios of CH₂ peak, relative to the unsuppressed water resonance (as percentage).

Results: RQ was significantly lower in the fasted condition compared with the glucose-supplemented condition during both exercise and recovery from exercise (0.77 ± 0.01 vs 0.85 ± 0.02 , respectively, $p=0.007$). Fat oxidation was significantly higher in the fasted condition compared with the glucose-supplemented condition (385.5 ± 108.3 mg/min vs 281.0 ± 87.5 mg/min, respectively, $p=0.004$). In the fasted condition IHL content was elevated directly after exercise and four hours post-exercise compared with baseline (from 1.72 ± 0.33 % at baseline to 1.91 ± 0.36 % post-exercise, $p=0.03$, to 2.06 ± 0.32 % four hours post-exercise, $p=0.023$), whereas it did not change when glucose supplementation was given (from 1.69 ± 0.32 % at baseline to 1.77 ± 0.36 % post-exercise, $p=0.30$, to 1.63 ± 0.35 % four hours post-exercise, $p=0.30$).

Conclusion: Acute exercise in the fasted state increased rather than decreased IHL content. However, IHL content did not change during exercise when the elevation of plasma FA in response to exercise in the fasted state was blunted by glucose intake. These data suggest that plasma FA concentrations play an important role in determining IHL content, and that a single bout of exercise may not be able to lower IHL.

Clinical Trial Registration Number: NCT01177332

Supported by: DFN

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Molecular basis of nonalcoholic fatty liver disease (NAFLD) in obese patients with or without glucose intolerance

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Background and aims: The basis of hepatocellular injury and progressive fibrosis in patients with obesity, Type 2 Diabetes and with nonalcoholic fatty

liver disease (NAFLD) is poorly understood. We sought to identify hepatic factors that are differentially abundant across the histologic spectrum of NAFLD.

Materials and methods: 45 non diabetic obese patients undergoing bariatric surgery (sleeve gastrectomy or gastric bypass) were characterized before the intervention with oral glucose tolerance, euglycemic hyperinsulinemic clamp, lipid and inflammatory biomarkers. Two biopsies were performed during the intervention, one for histological analysis and one for RNA and peptides extraction. RNA was extracted for differential gene expression with Affimetrix human GeneChips U 133 plus 2.0. Hepatic peptides were analyzed on an Q-TOF Premiere mass spectrometer with mass lynks/protein lynks software. 1-tail or 2-tail T tests were performed and used to screen for differential abundance gene expression or peptides expression, respectively.

Results: Patients were divided in four groups according to glucose tolerance status and NAFLD/NASH score: (1) obese with normal glucose tolerance/simple steatosis (NGT/SS, n=16), (2) obese with normal glucose tolerance/nonalcoholic steatohepatitis (NGT/NASH, n=7), (3) obese with type 2 diabetes/simple steatosis (DM2/SS, n=7), and (4) obese with type 2 diabetes/NASH (DM2/NASH, n=15). Comparative gene expression and protein abundance analyses were performed in a subset of DM2/NASH (n=3) and NGT/SS (n=3) patients. Most significant hits were controlled in the other samples by rtPCR and western blots. Gene expression analysis revealed that most significant annotation clusters were fatty acid biosynthesis, nucleosome core and response to hormone stimulus. Among most significant upregulated genes in DM2/NASH were: fatty acid binding protein 4, protein kinase AMP-activated alpha 2 catalytic subunit, neurotrophin Y receptor Y6 (pseudogene), CD36 molecule, serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1) member 1. Most significant upregulated genes in NGT/SS were: complement component 1s subcomponent, interleukin 6 signal transducer (gp130), itchy E3 ubiquitin protein ligase homolog, salt-inducible kinase 1, pyruvate dehydrogenase kinase isozyme 4. Shotgun Proteomics revealed that 7 known proteins were significantly expressed with differential abundance between study groups. In DM2/NASH liver were highly expressed Cytochrome P450 2C19 GN (CYP2C19), Phosphoglycerate kinase 2 GN (PGK2) and Aldo-keto reductase family 1 member C4 GN (AKR1C4). We also found that Microsomal triglyceride transfer protein large subunit GN (MTTP), Glutathione S-transferase Mu 1 GN (GSTM1), Lumican GN (LUM) and Prelamin-A/C GN (LMNA) were highly represented in NGT/SS liver biopsies.

Conclusion: Our data suggest that the pathogenesis of liver inflammation and fibrosis in obese insulin resistant patients with histologically progressive NAFLD is associated to multiple pathways and particularly to a deregulation of lipid metabolism.

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Interaction of lipid accumulation with mitochondrial function during the development of hepatic steatosis

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Background and aims: In muscle, reduced ATP production relates to insulin resistance and fat storage. Recently, hepatic ATP production was also found to be lower in humans with type 2 diabetes and non-alcoholic fatty liver (NAFL). However, it is unclear whether hepatic energy metabolism may be impaired prior to the development of NAFL and thereby directly affects mitochondrial function. To address this question, we studied two transgenic mouse strains which develop NAFL due to liver- (alb-SREBP-1c: ALB) or adipose tissue- (ap2-SREBP-1c: AP2) specific overexpression of the lipogenic transcription factor, sterol regulatory-element binding protein-1c (SREBP-1c), and wild-type mice (CON) under unfasted (n: ALB=3, AP2=3, CON=7) and fasting conditions (n: ALB=4, AP2=5, CON=3). Alb-SREBP-1c mice develop primary NAFL due to stimulation of hepatic lipogenesis, while ap2-SREBP-1c mice exhibit lipodystrophy and excessively store liver fat resulting in secondary NAFL.

Materials and methods: Mitochondrial oxidative capacity (complex I and II) was measured by high-resolution respirometry (Oroboros Instruments) in permeabilized liver samples after addition of 2 mM malate, 10 mM pyruvate, 10 mM glutamate, 2.5 mM ADP and 10 mM succinate. Mitochondrial content was determined from the ratio of mtDNA to nuclear DNA, using quantitative

PCR. Serum insulin concentrations were determined fluorometrically by ELISA (Mercodia). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was computed from fasting glucose and insulin concentrations. **Results:** At 18 weeks, AP2 are lipodystrophic and have 37% greater ($p<0.001$) liver weight than in ALB and CON. Their livers showed doubled ($p<0.004$) state-3 mitochondrial oxidation rates of complex I and II compared to ALB and CON (AP2: 63 ± 7 , ALB: 31 ± 8 , CON: 31 ± 9 pmol.s⁻¹.mg⁻¹). On the other hand, hepatic mitochondrial content of AP2 was 17% lower than in ALB and CON (1.31 ± 0.03 , 1.57 ± 0.11 , 1.57 ± 0.08 ; $p<0.008$ vs. ALB and CON). In AP2, postprandial serum insulin concentrations were 3.2fold higher than in ALB and 6.8fold higher than in CON (AP2: 776.0 ± 89.4 , ALB: 243.8 ± 282.5 , CON: 113.4 ± 67.9 pmol/L; $p<0.001$ AP2 vs. CON; $p=0.003$ AP2 vs. ALB). HOMAR-IR indexes derived from fasting glucose and insulin levels revealed severe insulin resistance in AP2 (5.8 ± 2.0 , ALB: 2.2 ± 1.2 , CON: 1.0 ± 0.6 ; $p<0.02$ AP2 vs. ALB, $p<0.01$ AP2 vs. CON).

Conclusion: Adipose-tissue specific overexpression of SREBP-1c causes lipodystrophy with subsequent development of steatosis and insulin resistance. This is associated with lower content, but greater oxidative capacity of liver mitochondria and could result from elevated intrahepatic lipid supply and/or compensatory increased mitochondrial function.

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Increased endogenous formation of N^ε-(Carboxymethyl)lysine in fatty liver induces inflammatory markers in an *in vitro* model of hepatic steatosis

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Background and aims: (Nonalcoholic fatty liver disease) NAFLD is strongly associated with the presence and severity of obesity, and are associated with a high risk of developing insulin resistance and type 2 diabetes. Increased lipid peroxidation and cytokine-induced inflammation are major pathways in the pathogenesis of NAFLD. An important lipoxidation product that could play a role in the induction of hepatic inflammation is the biologically active advanced glycation/lipoxidation endproduct N^ε-(Carboxymethyl)lysine (CML). Therefore, the aim of the present study was to investigate the relationship between steatosis and the accumulation of CML, and to study the role of CML in hepatic inflammation.

Materials and methods: This study included 31 severely obese individuals, which were categorized into 3 groups according to the grade of hepatic steatosis. CML accumulation in liver biopsies was assessed by immunohistochemistry, and CML plasma levels were measured by ultra performance liquid chromatography-tandem mass spectrometry. CML plasma levels were also determined in a second cohort of 22 individuals with a range of body mass index (BMI). In these individuals, CML levels in the hepatic artery, portal vein and hepatic vein were determined and CML fluxes across the liver were calculated by multiplying arterialized-venous concentration differences with hepatic plasma flow. Hepatocyte HepG2 and HuH7 cell lines were used to study endogenous CML formation during intracellular lipid accumulation. Real time PCR analyses were performed to investigate the effects of CML on the expression of pro-inflammatory cytokines in cultured hepatocytes.

Results: CML accumulation in the liver was significantly higher in individuals with moderate and severe grade of steatosis compared with individuals with a low grade of steatosis (IHC score 2.0 ± 0.1 vs 1.2 ± 0.1 , $p<0.001$, and 1.9 ± 0.5 vs 1.2 ± 0.1 , $p=0.023$, respectively). Analysis of CML fluxes across the liver indicated that there was no release or uptake of CML by the liver. Intracellular lipid accumulation in cultured hepatocytes, as induced by incubation with a mixture of linoleic and oleic acids, was associated with increased endogenous CML formation in HepG2 and HuH7 cells (21% and 42% increase in CML levels, respectively, $p<0.05$ for both), which was accompanied by increased gene expression of receptor for advanced glycation endproducts (RAGE), plasminogen activator inhibitor-1 and interleukin-6. Endogenous CML formation and the increased gene expression were inhibited by pyridoxamine and aminoguanidine, both inhibitors of ALE formation.

Conclusion: There is a significant accumulation of CML in steatotic livers of obese individuals. The CML-induced inflammation in fatty livers may play a role in the pathogenesis of NAFLD and/or in the increased high risk of developing insulin resistance and type 2 diabetes.

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Apolipoprotein C-I concentration is associated with liver fat content and triglyceride levels in patients with type 2 diabetes

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Background and aims: Apolipoprotein (Apo) C-I mainly affects lipoprotein metabolism by inhibiting Lipoprotein Lipase (LPL) and promoting Very Low Density Lipoproteins (VLDL) production. In accordance with observations in mouse models, high Apo C-I levels have been shown to be associated with increased triglyceride (TG) concentrations and decreased visceral fat mass in men with metabolic syndrome. Non alcoholic fatty liver disease is commonly associated with obesity, metabolic syndrome and type 2 diabetes (DT2). So far, Apo C-I has never been studied in DT2. This prompted us to determine whether Apo C-I concentrations are associated with TG levels, visceral fat area and liver fat content in patients with DT2.

Materials and methods: Liver fat content (1H-magnetic resonance spectroscopy), area of visceral fat (single slice axial T1-RMN), Apo C-I and TG concentrations were measured in 121 patients with type 2 diabetes. High Apo C-I concentration was determined according to median Apo C-I concentration (77.4 mg/L).

Results: TG concentrations (2.85 ± 2.12 vs 1.85 ± 1.11 g/L, $p=0.001$) and liver fat content (13.51 ± 9.63 vs $9.73 \pm 8.3\%$, $p=0.02$) were significantly higher in patients with high Apo C-I compared with low Apo C-I. The visceral fat area was borderline significantly increased (266.71 ± 93.52 vs 242.71 ± 103.46 cm², $p=0.088$) in subjects with high Apo C-I levels compared with low Apo C-I. There was no correlation between Apo C-I concentrations and BMI and between ApoC-I levels and subcutaneous adipose tissue. In multivariate analyses, liver fat content (>median level:9.98%) was associated with Apo C-I (> median concentration:77.4 mg/L) ($p=0.01$), TG ($p=0.0287$) and BMI ($p=0.002$), but not with age and sex.

Conclusion: The association between high Apo C-I concentrations and decreased visceral fat described in patients with metabolic syndrome is not observed in type 2 diabetic patients. High Apo C-I levels are correlated with high TG levels and liver fat content in type 2 diabetic patients. Liver fat content is independently associated with Apo C-I. It may be explain by inhibition of LPL, which may increase TG concentrations and promote hepatic TG storage. Further studies are needed to evaluate cross-links between Apo C-I and fatty liver.

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Regulation of hepatic lipid and cholesterol synthesis by PEPCK-C

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Background and aims: Cytosolic Phosphoenolpyruvate carboxykinase (PEPCK-C) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate. Although this step is classically considered gluconeogenic, PEPCK-C is also expressed in non-glucose producing tissues like adipose tissue and is more generally a cataplerotic pathway required to match mitochondrial anaplerosis to biosynthesis and dynamic regulation of the TCA cycle. In addition to gluconeogenesis (GNG), PEPCK-C is important for glyceroneogenesis (GyNG), triglyceride (re)esterification and mitochondrial function. Despite being required for GNG, we previously demonstrated that the control coefficient of PEPCK-C over hepatic GNG is surprisingly low (0.18), but that complete loss of liver PEPCK-C causes impaired mitochondrial function. Liver-specific PEPCK knockout mice have a remarkable increase in hepatic triglyceride levels after an overnight fast, further underscoring the importance of this enzyme in the regulation of lipid metabolism. GyNG is a pathway that shares most enzymatic reactions with GNG and is critical for triglyceride synthesis. The objective of this study was to determine the consequence of PEPCK-C loss of function on the regulation of GyNG and its broader impact on lipid metabolism.

Materials and methods: Three types of mice with graded expression of PEPCK-C were used in this study: control (pck-1 lox/lox), 70% (pck-1 lox/neo) and 90% (pck-1 lox + neo/del) whole body knockdown and a liver specific PEPCK Knockout (pck-1 lox/lox+AlbCre). Mice were I.P. injected

with 27 μ l/g 99.9% D₂O to achieve a body water enrichment of 4% and then given 4% deuterated drinking water and chow ad libitum for 4-days to label hepatic and adipose triglycerides and cholesterol. Tissue triglycerides were extracts via Folch extraction and purified via solid phase extraction before being analyzed by ¹H and ²H nuclear magnetic resonance (NMR). Synthesis of the glycerol and fatty acid moieties of triglycerides and cholesterol were determined from incorporation of deuterium compared to total deuterium body water enrichment.

Results: There was a direct correlation between synthesis fluxes and whole body PEPCK-C expression (see table). Remarkably, this correlation suggested a stronger control of fed state GyNG by PEPCK-C than we previously reported for fasted state GNG. Despite no obvious requirement for PEPCK-C in lipogenesis or cholesterologenesis, the synthesis of total FFA, unsaturated FFA and cholesterol strongly correlated with levels of PEPCK-C. This observation was most apparent in whole body PEPCK-C deficient mice and less evident in liver specific KO mice.

Conclusion: These data confirm an important role for PEPCK-C in glycerol and triglyceride metabolism and reveal an unexpected role for PEPCK-C in fatty acid and cholesterol synthesis

Hepatic fluxes in animals with graded PEPCK. Data presented mean \pm SEM. * p < 0.05 vs control

	lox/lox 100% PEPCK-C	lox/neo 30% PEPCK-C	lox + neo/del 10% PEPCK-C	lox/lox+AlbCre 0% PEPCK-C
Fatty acid synthesis (mg/day/g of liver)	13.93 \pm 1.09*	8.39 \pm 1.23*	6.39 \pm 0.53*	9.65 \pm 1.79*
glycerol synthesis (mg/day/g of liver)	7.35 \pm 0.97	6.49 \pm 1.13	4.14 \pm 0.71*	2.84 \pm 0.33*
Unsaturated fatty acid synthesis (mg/day/g of liver)	5.00 \pm 0.61	2.08 \pm 0.41*	1.73 \pm 0.16*	2.99 \pm 0.52*
Cholesterol synthesis (mg/day/g of liver)	115.22 \pm 17.78*	59.66 \pm 12.73*	43.78 \pm 7.53*	97.82 \pm 4.82*

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Obesity influences white matter integrity: a combined diffusion tensor imaging and voxel-based morphometric study

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Background and aims: Obesity has profound impact on several organs, yet little is known about its effect on brain structure. In this study we wanted to test whether morbid obesity is associated with structural changes in the brain's white and gray matter integrity.

Materials and methods: We compared brains of 23 neurologically intact, morbidly obese subjects and 22 neurologically intact, age-matched normal-weight volunteers with diffusion tensor imaging (DTI) and voxel-based morphometry (VBM) of T1-weighted anatomical magnetic resonance imaging (MRI) images. Full-volume statistical parametric mapping (SPM) analysis was used to compare fractional anisotropy (FA) and mean diffusivity (MD) values as well as gray and white matter density between the obese and normal-weight groups.

Results: Obesity was associated with lowered FA and MD values as well as reductions in both focal and global gray and white matter volumes. The focal obesity-related structural changes were observed in brain regions governing reward seeking, inhibitory control and appetite. Regression analysis showed that focal FA and MD values as well as gray and white matter density in these regions were negatively associated with body fat percentage. However, correlations with blood pressure, triglycerides, LDL, HDL or HbA_{1c} were mostly nonsignificant.

Conclusion: Anatomical changes in the gray and white matter in the obese persons' brains may partly be due hormonal and metabolic factors. These focal reductions were observed in brain regions involved in reward processing and appetite control, thus they may further promote abnormal reward seeking and eating behavior in obese individuals. Our results suggest that in order to prevent overeating in obese individuals, both behavioral and pharmacological treatments for obesity should aim at modifying the functional properties of the reward and cognitive control circuitry.

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Cerebral glucose metabolism in human type 1 diabetes is increased after treatment with insulin detemir compared to NPH insulin: possible explanation for differences in weight?

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Background and aims: The aim of this study was to test the hypothesis that insulin detemir, which consistently has been shown to result in less weight gain than other insulin therapies, leads to a more pronounced effect on brain glucose metabolism than NPH insulin in human type 1 diabetes.

Materials and methods: Eighteen male type 1 diabetic patients (mean \pm SD; age 38.5 \pm 8.9 years, body weight 83.1 \pm 13.5 kg, BMI 24.7 \pm 2.6 kg·m⁻², diabetes duration 14.0 \pm 7.5 years, HbA_{1c} 7.5 \pm 0.7%) were included in a randomised cross-over study. Patients were treated with a basal bolus regimen for 2 periods of 12 weeks, starting with either insulin detemir or NPH insulin, in combination with insulin aspart. After each treatment period, all patients were scanned in the morning in the fasted state; patients had injected their last basal insulin the previous night. Dynamic [¹⁸F]-fluoro-2-deoxy-D-glucose (FDG) brain scans were acquired over 60 minutes using an HRRT (Siemens/CTI) PET scanner. Arterial blood was measured continuously using an on-line sampling system. Using a standard template, various brain regions

were defined automatically on an MRI scan. Cerebral metabolic rate of glucose uptake (CMRGlu) was obtained by non-linear regression of the regional time-activity curves using the standard irreversible 2-tissue compartment model with blood volume parameter together with an arterial plasma input function. A lumped constant of $0.83322 - 0.0043 \times (\text{arterial glucose level})$ was used, based on previous human and rat data. Differences between treatments were tested by paired 2-tailed t-tests.

Results: After 12 weeks, daily insulin doses and HbA1c were similar between treatment groups. Insulin detemir decreased body weight by 0.3 kg, whereas NPH insulin increased weight by 0.5 kg (between-group difference $p=0.18$). After treatment with insulin detemir, average brain CMRGlu was increased with 4.7% when compared to NPH insulin ($p<0.01$); this effect was consistently shown in all brain regions including those regions involved in appetite regulation. Arterial glucose levels during scanning were similar between treatments.

Conclusion: Treatment with insulin detemir versus NPH insulin resulted in an increase in cerebral glucose metabolism in men with type 1 diabetes, paralleled by a trend towards weight loss. These findings support the hypothesis that a differential effect on cerebral glucose metabolism may contribute to the consistently observed weight sparing effect of insulin detemir.

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Does central dopaminergic activity influence metabolic parameters?

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Background and aims: Dopaminergic agonists have been proved to improve control of diabetes and lipids. Central dopaminergic activity can be measured by apomorphin challenge test (apomorphin increases growth hormone and decreases prolactin levels), which is a marker of dopaminergic activity. The aim of our study was to evaluate the relationship between central dopaminergic activity and metabolic parameters in healthy men.

Materials and methods: We examined 42 healthy men (average age 43.5 ± 7.4 years, BMI $27.4 \pm 5.7 \text{ kg/m}^2$), anthropometric (waist-hip ratio, blood pressure and body fat by bioimpedance) and metabolic (glycaemia, lipids, glycated hemoglobin) parameters were measured and HOMA index of insulin resistance was calculated at the beginning of the study. Sublingual apomorphine (0.033 mg/kg with 4 mg as the highest dose) was administered and basal prolactin and growth hormone were measured in -30, -15, 0, 15, 30, 45, 60, 75, 90, 120, 150, 180 minutes. Area under the curve for prolactin (AUCPRL) and growth hormone (AUCGH) was calculated using trapezoidal rule. Euglycemic hyperinsulinemic clamp (insulin 1 mg/kg/min) was performed and glucose disposal (M) calculated. Linear regression was used for statistical analysis.

Results: Negative correlation was observed between AUCPRL resp. AUCGH and total cholesterol ($r=-0.52$, $P=0.001$; resp. $r=-0.34$, $P=0.04$), triglycerides ($r=-0.42$, $P=0.009$; resp. $r=-0.36$, $P=0.03$), HOMA index ($r=-0.33$, $P=0.043$; resp. $r=-0.5$, $P=0.001$), percentage of body fat ($r=-0.44$, $P=0.009$; resp. $r=-0.45$, $P=0.007$) and BMI ($r=-0.36$, $P=0.013$; resp. $r=-0.56$, $P=0.0001$). Negative correlation was also found between AUCGH and glycated hemoglobin ($r=-0.55$, $P=0.0001$), AUCGH and diastolic blood pressure ($r=-0.35$; $P=0.039$), waist hip ratio ($r=-0.46$; $P=0.006$) and age ($r=-0.53$; $P=0.001$), whereas positive correlation between AUCGH and M ($r=0.36$, $P=0.039$). After adjustment for age and BMI negative correlation between AUCGH and HbA1c ($r=-0.37$, $P=0.016$), AUCGH and HOMA index ($r=-0.34$, $P=0.025$), resp. AUCPRL and total cholesterol ($r=-0.41$, $P=0.007$) remained statistically significant.

Conclusion: Lower central dopaminergic activity is connected with higher total cholesterol, glycated hemoglobin and HOMA index after adjustment for BMI and age in healthy men.

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Impact of milk fat- and canola oil-enriched diets on peripheral glucose metabolism and insulin action in the mouse brain

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Background and aims: Increased consumption of dietary fats and reduced physical activity are the most important environmental factors leading to obesity, insulin resistance and finally type 2 diabetes. In this respect, the source and structure of dietary fat plays an important role, and in vitro studies suggested that saturated fatty acids (SFA) impair insulin action and secretion while mono-unsaturated fatty acids (MUFA) might be beneficial. Along with alterations in insulin action and secretion in the periphery, the brain emerged as a target for SFA to impair insulin action and neuronal activity. Moreover, insulin resistance in the brain was linked to an increase in food intake, peripheral insulin resistance and physical inactivity. The aim of the study was to analyze the effect of dietary SFA versus MUFA in vivo in mice.

Materials and methods: Four-week old C57BL/6 mice were weaned on an isocaloric milk fat- or canola oil-enriched diet with a total fat content of 8.3% and on chow diet for 8 weeks and the impact on glucose tolerance, insulin sensitivity and secretion, as well as cortical (ECOG) and locomotor activity, assessed by radiotelemetric measurements, was evaluated. Magnetic resonance imaging (MRI) of total and visceral fat tissue was performed on a 3 T whole-body imager.

Results: Both groups of mice with fat-enriched diet displayed a significant increase in body weight compared to the control group which was accompanied by impaired locomotor activity. MRI measurements revealed a significant increase in visceral adipose tissue mass (vs. control: milk: $+134.3\%$, $p<0.001$; canola: $+85.3\%$, $p<0.05$). The increase in body fat was accompanied by an impairment in glucose tolerance and insulin secretion and increased plasma non-esterified fatty acid concentrations (milk: 740 ± 48 vs. control: $588 \pm 46 \text{ } \mu\text{mol/l}$, $p<0.05$) in the milk fat-enriched diet group but not in the canola fat group. Interestingly, radiotelemetry analysis revealed a significant decrease in cortical activity in the milk fat group while canola oil even improved cortical activity. In addition, an intracerebroventricular application of human insulin was accompanied by an increase in locomotor activity in canola oil- and chow-fed mice, while mice fed the milk fat-enriched diet displayed insulin resistance. In order to study molecular mechanisms of altered cortical activity by the two fat-enrichments, tyrosine phosphorylation of the insulin receptor (IR) was assessed in the brain after intravenous injection of human insulin. Thereby, an increase in insulin-mediated tyrosine phosphorylation of the IR in total brain lysates was detected in the chow and canola-fed animals, but absent in milk-fat fed mice.

Conclusion: Feeding of mice with either canola oil that consists predominantly of MUFA or milk fat characterized by a large amount of SFA significantly increased body weight and body fat mass compared to chow. Moreover, both diets are accompanied by a significant decrease in physical activity. However, glucose tolerance and insulin secretion were solely impaired in the milk fat group but unaffected in the canola fat group and this was also true for measures for insulin resistance in the brain. Therefore, upon a moderate isocaloric intake of dietary fat, SFA display aversive effects on insulin secretion and the brain while MUFA were protective.

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A mouse model of Alzheimer's disease develops insulin resistance

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Background and aims: Type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) share many common features such as common susceptibility genes, production of amyloid plaques and predisposition with age. The risk to develop AD is double in patients with T2DM. Furthermore, DM may induce micro and macrovascular complications on the brain, a risk factor for AD. In parallel it has been reported that AD's predisposition can be linked to T2DM development. Lepr^{-/-} (db/db) mice develop obesity, hyperinsulinemia and hyperglycemia, being considered a model of obesity, insulin resistance and type 2 diabetes. Lepr^{+/-} mice do not show any metabolic phenotype, so

they are used, together with *lepr*^{+/+}, as a control group when compared to the *lepr*^{-/-}. APPsw mice overexpress amyloid precursor protein (APP) which is processed into Aβ peptide, these mice have detectable levels of Aβ on their plasma and they are used as a model of AD.

Materials and methods: We were interested in whether circulating high levels of Aβ can have an effect on glucose metabolism. We characterized the metabolic phenotype of transgenic APPsw versus wild type (WT) mice, measuring weight, blood glucose, plasma insulin, glucose tolerance and insulin response to glucose challenge *in vivo*.

Results: First we characterized 5–7 weeks old mice (APPsw versus WT) and found they are both, normoglycemic and normoinsulinemic, showing normal glucose tolerance test. Then, we decided to include a genetic risk to develop diabetes, breeding *lepr*^{+/+} mice with APPsw, generating *lepr*^{+/+} mice having the APPsw transgene (*db*^{+/+}; Tg-APP) or not (*db*^{-/-}; WT). We then characterized the metabolic phenotype of these mice, finding unexpectedly that *db*^{+/+}; Tg-APP were normoglycemic but hyperinsulinemic in non-fasting conditions and glucose intolerant, showing a phenotype of insulin resistance. Their hyperinsulinism is not mediated by improved beta-cell function, as their insulin levels 15 minutes after glucose challenge are similar in *db*^{+/+}; Tg-APP and in *db*^{+/+}; WT mice.

Conclusion: Thorough investigation will be performed for a better understanding of the phenotype (insulin tolerance test, pyruvate tolerance test, beta-cell mass) as well as mechanistic studies to decipher if insulin resistance is due to circulating APP or if it is secondary to alterations in the central nervous system (CNS). This study will shed light into the mechanisms underlying AD's link to T2DM, and it will give clues to the hypothesis that the individuals bounded to suffer the first disease will be predisposed to suffer the second one and vice versa.

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Insulin 2 is present in the adult mouse brain and its gene expression controls food intake

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Background and aims: The murine insulin 1 and insulin 2 genes are widely considered to be functionally redundant, despite reports of distinct tissue distribution, differential regulation in the embryo and distinct promoter elements. While it is obvious that pancreatic β-cells are the only source of circulating insulin, reports throughout the years have pointed to extra-pancreatic insulin production and local action (e.g. thymus, brain). The brain is a known target for insulin's effects on satiety and energy homeostasis. The goal of the present study was to further test the hypothesis that insulin 2 is selectively expressed in the adult brain and determine the effect of graded insulin 2 gene expression on high-fat food intake and obesity. We hypothesize that adult brain insulin 2 normally suppresses fatty food intake and peripheral insulin sensitivity, and that reductions in brain insulin 2 can cause obesity.

Materials and methods: We employed Taqman RT-qPCR with insulin 1 or insulin 2 knockout mice as controls to examine the mRNA expression of insulin 2 in multiple parts of the brain. We tested the effect of control and 58% high-fat diets on multiple parameters, including obesity, glucose homeostasis and food intake in *ins1*^{-/-}; *ins2*^{+/-} mice with reduced insulin 2 and their *ins1*^{-/-}; *ins2*^{+/+} littermate controls. All groups in this study lacked the pancreas-specific insulin 1 gene.

Results: *Ins1*^{-/-}; *ins2*^{+/-} mice on a control diet had significantly lower insulin release when compared to *ins1*^{-/-}; *ins2*^{+/+} mice. This was associated with a significant reduction in body weight. -Cell mass and glucose homeostasis were similar in all groups. Mice with low insulin 2 gene dosage (*ins1*^{-/-}; *ins2*^{+/-}) were initially hypersensitive to insulin on normal chow, but eventually became paradoxically insulin resistant. *Ins1*^{-/-}; *ins2*^{+/-} mice showed increased body weight on a high fat diet relative to *ins1*^{-/-}; *ins2*^{+/+} controls. Food intake was selectively increased in high fat-fed *ins1*^{-/-}; *ins2*^{+/-} mice relative to *ins1*^{-/-}; *ins2*^{+/+} mice, suggesting a central mechanisms of weight gain, consistent with a satiety role for insulin 2. We also found brain insulin 2 gene expression was reduced by high fat feeding, a potential vicious cycle.

Conclusion: Together with our work on insulin 1, our data strongly suggest that the insulin 1 and insulin 2 have distinct functions in body weight regulation and energy homeostasis. Our data clearly show insulin 2 in the brain. Local brain insulin 2 may play an amplified satiety role in the absence of insulin 1. Indeed, the *ins1*^{-/-}; *ins2*^{+/-} mice resemble brain-specific insulin receptor knockout mice, showing exaggerated obesity with a high fat diet. The expres-

sion of two murine insulin genes presents a unique opportunity to genetically dissect the tissue-specific roles of insulin 1 (pancreas) from the actions of insulin 2 (pancreas + brain). Since all mammals, including humans, express insulin in tissues outside the pancreas, our studies have the potential to help us understand the tissue- and diet-dependent effects of insulin on obesity and energy homeostasis.

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Serum brain-derived neurotrophic factor (BDNF) concentration is down-regulated by Intralipid/heparin infusion in young and healthy male subjects

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Background and aims: Insulin resistance and type 2 diabetes are associated with an increased risk of neurodegenerative diseases. Brain-derived neurotrophic factor (BDNF) regulates neuronal differentiation and synaptic plasticity and its decreased levels are supposed to play a role in the pathogenesis of Alzheimer disease and other disorders. The aim of the present study was to estimate the effects of hyperinsulinemia and plasma NEFA elevation on serum BDNF concentration in humans.

Materials and methods: We studied 18 healthy male subjects (mean age 25.61±3.01 years; mean BMI 26.62±4.76 kg x m⁻²). Serum BDNF concentration was measured in the baseline state and in the 120 and 360 minutes of euglycemic hyperinsulinemic clamp with or without Intralipid/heparin infusion.

Results: There was more than 3-fold increase in plasma NEFA from the initial values during Intralipid/heparin infusion (*p*<0.001). Insulin sensitivity was not different during the second hour of both clamps (*p*=0.47), however, it was reduced by approx. 40% after six hours of Intralipid/heparin infusion (*p*<0.001). Hyperinsulinemia had no effect on serum BDNF concentration (*p*=0.89). Raising NEFA had no effect on serum BDNF in 120 minute (*p*=0.99), however, it resulted in a significant decrease by 43% in serum BDNF value after 360 minutes (*p*=0.005). Additionally, BDNF 360 min value of Intralipid/heparin was markedly lower in comparison with BDNF 360 minute value of the clamp without raising NEFA (*p*=0.007). Serum BDNF in 360 minute of the clamp was related to a decrease in insulin sensitivity during Intralipid/heparin infusion (*r*=−0.50, *p*=0.035).

Conclusions: Our data show that raising NEFA decreases serum BDNF. This might indicate a potential link between NEFA-induced insulin resistance and neurodegenerative disorders.

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PS 054 Adipose structure and stem cells

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Type 2 diabetes alters the stem cell content of the stromal vascular fraction of subcutaneous adipose tissue

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Background and aims: Cell therapy is a promising option for treating ischemic diseases and heart failure. Nevertheless, it has recently been shown that aging and cardiovascular risk factors, such as diabetes, affect endogenous progenitor cells thereby limiting their therapeutic potential. The impact of diabetes on the number and function of endothelial progenitor cells (EPCs) and bone marrow derived progenitor cells (BMSC) is already known. However, its impact on other stem cell populations remains unclear. Recently, different tissues have emerged as possible stem cell sources, and adipose tissue represents a particularly abundant, practical, and appealing source for autologous cell replacement. Therefore, the aim of this study was to investigate the effects of diabetes on stem cell content in the adipose stromal vascular fraction (SVF).

Materials and methods: Subcutaneous adipose tissue from Zucker Diabetic Fatty (ZDF) and lean normoglycemic control (ZDFc) rats was dissected and processed for SVF isolation. Total RNA from the SVF was isolated and gene expression was analyzed by a specific Rat Stem Cell PCR Array. Additionally, total RNA from subcutaneous adipose tissue was isolated and specific stem cell markers expression were measured by real-time PCR.

Results: ZDFc rats showed normal blood glucose levels (92 ± 1.99 mg/dl) whereas those of ZDF rats were significantly higher (392.75 ± 11.62 mg/dl, p -value <0.0001 vs. lean controls) clearly reflecting their diabetic status. Real-time PCR analysis of subcutaneous adipose tissue showed a reduction of expression of stem cell markers CD90 (42.01 ± 6.38 %), CD29 (76.81 ± 9.51 %) and CD105 (71.32 ± 7.85 %) in ZDF compared to controls. However, only CD90 and CD105 showed a significant difference (p -value 0.0003 and 0.0289, respectively). The PCR Array used analyses expression of genes related to stem cell identification and growth. Diabetes was associated with an overall down regulation of stem cell specific markers in SVF (a cut off fold change of 1.5 was applied). Interestingly, mesenchymal cell lineage markers (Acan, Alpi, Bglap, Col1a1, Col2a1 and Col9a1), genes involved in signalling pathways important for stem cell maintenance (Notch and Wnt pathway) and genes involved in maintaining pluripotency and self-renewal status (Fgf4, Gdf3, Sox2 and Tert) showed a reduced expression in the SVF from diabetic rats.

Conclusion: These findings suggest that type 2 diabetes affects the maintenance of the "stemcellness" characteristics of stem cells and their content in the SVF of subcutaneous adipose tissue. Impairment of diabetic-derived progenitor cells might limit the efficiency of direct autologous stem cell therapy with SVF. Further isolation from stroma and characterization of these stem cells is required to truly confirm their therapeutic potential.

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Early transcriptional activation of adipose genes precedes the phenotypic remodelling of adipose tissue in pregnancy

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Background and aims: Pregnancy is characterized by significant changes in basal maternal metabolic homeostasis with extensive adipose tissue (AT) remodeling. Early gestation is viewed as an anabolic state with increasing maternal adipose stores to meet the feto-placental and maternal energy demands at later time. The aim of this study was to characterize the mechanisms responsible for adipose tissue remodelling focusing on the molecular events occurred in adipose tissue during the early stage of pregnancy.

Materials and methods: 11 women with normal glucose tolerance were recruited pre-gravid and followed-up longitudinally in early (8-12 weeks) pregnancy. The metabolic profile, body composition and insulin sensitivity were obtained at pre-pregnancy (P) and again at early pregnancy (E). White adipose tissue biopsies were obtained by liposuction in the subcutaneous gluteal depot at time P and E. Modification of adipose tissue transcriptome

were assessed by microarray (U133A2 Affymetrix) and RT-PCR of candidate genes.

Results: BMI, fat mass, adipose cell volume and cellularity and insulin resistance index were all significantly increased at L ($p < 0.001$) and unchanged at E compared to P. Among the 22,278 genes surveyed, the adipose tissue transcriptome encompassed 7612 ± 524 genes. At time E 15% of the adipose transcriptome (1286 genes) was significantly modified (645 increased and 641 decreased) and 10 % at time L. Stringent filtering (p value < 0.001 and $FC > \pm 1.5$) and hierarchical cluster analysis identified 4 main functional categories overrepresented in early pregnancy: metabolism related genes, ECM, immune regulation and angiogenesis. In the lipid cluster increased in ACLY-ATP citrate lyase, Stearoyl-CoA desaturase, Fatty acid synthase indicated enhancement of lipogenesis. There were an increase in genes regulating angiogenesis (VEGFA, IGF2-, MMP-14, fibronectin) and recruitment of LPS-sensing pathways (lipopolysaccharide binding protein, CD14-, Toll-like receptor 4, NFKB).

Conclusion: Early pregnancy is characterized by a combination of molecular events which precede the phenotypic changes of adipose tissue and body composition. The activation of immune and angiogenic regulation are novel findings suggesting that early inflammation and vascular growth prepare for adipose tissue remodeling at later stages of pregnancy.

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Pericardial adipose tissue and stem cells in diabetics

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Background and aims: Several studies have shown adipose tissue plays an important role in endocrine regulation and as a source of mesenchymal stem cells (MSC) which might be involved in endogenous repair of organ damage. It has been suggested that pericardial adipose tissue (PAT) might interact with the vessel wall and regulate coronary artery disease progression. We hypothesized that diabetes can affect PAT-MSCs and reduce or modify their endogenous repair potential. In this study we aimed to investigate differences in PAT of ZDF (diabetic) and ZDFc (normal) rats in terms of their stem cell characteristics.

Materials and methods: PAT from ZDF and ZDFc rats was surgically excised and the stromal vascular fraction (SVF) isolated. Total RNA from SVF was extracted and gene expression analyzed by a specific Rat Stem Cell Array. The statistical analysis was realized with RT² Profiler™ PCR Array Data Analysis (SABiosciences). Biochemical analysis was performed following standard procedures.

Results: ZDF showed higher glucose levels than ZDFc (392.75 ± 11.62 mg/dl vs. 92 ± 1.99 mg/dl, p -value < 0.0001) at time of analysis (14th weeks). SVF-ZDF showed a significant reduction of cell content compared to control (p -value 0.01). Diabetic and control groups showed significant differences in gene expression with a general down-regulation trend in the diabetic rats. The most specific pluripotency markers (Tert, Sox2, Gdf3, Fgf4) were down-regulated and likewise several genes included in Notch and Wnt pathways (with important roles in stem cell maintenance). A few genes, involved in cell proliferation, were up-regulated in diabetic SVF (Cdc42, Col1a1, Fgfr1).

Conclusion: These results suggest that the number and pluripotency of stem cells in PAT are reduced in diabetes. Furthermore, genes involved in proliferation are up-regulated in diabetic PAT. These findings suggest that the increased progression of coronary artery disease found in diabetics may be associated to a lower endogenous repair capacity in PAT for organ damage.

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The effect on endoplasmic reticular stress in the liver and pancreas of prediabetes and diabetes stage OLETF rats after alcohol ingestion

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Background and aims: Diabetes mellitus is caused by beta cell loss or insulin resistance. Endoplasmic reticular stress (ER stress) mechanisms have been known to play a key role in developing diabetes. ER stress is caused by multiple factors, and alcohol is also one of them. The present study investigated the effect of alcohol on ER stress in the liver and pancreas of Otsuka Long Evans Tokushima Fatty (OLETF) rats.

Materials and methods: The OLETF rats aged 11–16 weeks were in the prediabetic stage. The prediabetic experimental group (15 OLETF rats) was treated with Liber DeCali regular ethanol-containing liquid diet. The prediabetic control group (15 OLETF rats) was treated with Liber DeCali regular control diet (35% fat, 47% carbohydrate, 18% protein). The each group was subjected to pair-fed methods with the same calories from 11 week to 16 week. In addition, the OLETF rats aged 17–22 weeks were in the diabetic stage. The OLETF rats in diabetic stage were given the same diet as prediabetic OLETF rats during 6 weeks. The diabetic experimental group and diabetic control group had 15 OLETF rats, respectively. An intraperitoneal - glucose tolerance test (IP-GTT) was conducted at the end of 16 weeks at the prediabetic stage and 22 weeks at the diabetic stage, respectively. Hematoxylin and eosin staining was performed to observe the morphological changes of liver and pancreatic tissues following alcohol treatment. To assess the degree of ER stress, western blot analysis was performed using ER stress markers.

Results: In the liver, alcohol treatment at the prediabetic stage did not induce the ER stress. The alcohol treatment in the diabetic stage showed an increase in p-PERK, phosphorylation-eukaryotic translation initiation factor 2 α (p-eIF2 α), activated transcription factor 6 (ATF6) and inositol requiring enzyme-1 α (IRE1 α) ($p < 0.05$). However, phosphorylation-c-Jun N-terminal kinase (p-JNK) associated with insulin resistance decreased in the liver at both prediabetic stage and diabetic stage. Meanwhile, in the pancreatic tissue of prediabetic stage, alcohol treatment increased significantly the expression of p-PERK, p-eIF2 α , ATF6, glucose regulated protein 78/binding immunoglobulin protein (Grp78/BiP) and p-JNK ($p < 0.05$). The morphology of the pancreatic islet was damaged. However, growth arrest and DNA damage-inducible gene C/EBP-homologous protein (GADD153/CHOP) in the alcohol group was not different from that of the control group. The morphology of the pancreatic islet was severely damaged at diabetic stage. The expression of p-PERK, p-eIF2 α , ATF6, CHOP, BiP, IRE1 α and p-JNK increased by excessive alcohol ($p < 0.05$).

Conclusion: The alcohol, regardless of the progression of the diabetes, affected the pancreas more than the liver. The compensation mechanism for alcohol-induced damage was operated in the liver but all the mechanisms of ER stress caused the pancreas to negative effects from alcohol. Therefore, the incidence of diabetes may be increased by alcohol consumption, regardless of the progression of diabetes.

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Methylglyoxal causes structural and functional alterations in adipose tissue independently of obesity

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Background and aims: Adipose tissue alterations including impaired adipocytokine expression, cell death, hypoxia and macrophage infiltration occur since early type 2 diabetes stages as it is the first organ to develop insulin resistance even on moderate BMI values. However, the contribution of hyperglycemia to these events during type 2 diabetes progression was never object of study. In this work we intended to evaluate the role of chronic hyperglycemia and specifically methylglyoxal (MG) on adipose tissue dysfunction.

Materials and methods: We studied normal Wistar (W) rats and a non-obese model of type 2 diabetes, Goto-Kakizaki (GK) rats with 6 and 14 months old, in order to understand chronic effects of hyperglycemia and ageing. We also studied a group of W rats with MG administration (WMG) in order to study MG-specific mechanisms. Several functional and morphological aspects were assessed, namely adipocytokines, fibrosis, glycation, hypoxia and inflammation.

Results: Hyperglycemia (fasting and 2 hours after i.p. glucose administration) is intrinsic to our diabetic animal model ($P < 0.001$) and resulted in structural adipose tissue alterations, namely fibrosis and accumulation of PAS positive components, exacerbated in aged animals. Although in a lesser extent, these alterations were also observed in aged W rats and in MG-treated group. Decreased adiponectin circulating levels ($P < 0.05$) were observed in aged diabetic and MG-treated rats. Diabetic rats, aged W and WMG showed decreased Bcl-2/Bax ratio ($P < 0.05$) and increased caspase 3 expression ($P < 0.05$). Diabetic rats, aged W and WMG also had impaired VEGF/Ang-2 ratio ($P < 0.05$). These alterations led to interstitial hypoxia quantified by pimonidazole staining ($P < 0.05$) and decreased tissue irrigation quantified by Evans Blue infiltration ($P < 0.05$). In aged diabetic rats hyperglycemia resulted in increased MCP-1 ($P < 0.05$) expression and macrophage (F4/80: $P < 0.05$) accu-

mulation in adipose tissue, localized in glycated fibrotic regions. These events were also observed in W rats treated with MG, but not in the other groups. **Conclusion:** Ageing causes several structural and functional modifications of adipose tissue also observable in early type 2 diabetes stages. However, chronic hyperglycemia-induced MG accumulation leads to macrophage recruitment for glycated fibrotic regions, common features of adipose tissue dysfunction models and obese or metabolic syndrome patients. Our results show that MG contributes to adipose tissue dysfunction during type 2 diabetes progression.

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Folate and vitamin B12 imbalance induces endoplasmic reticulum stress in human adipocytes

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Background and aims: Prospective longitudinal studies in humans show that low maternal B12 and high folate levels independently predict higher metabolic risk & insulin resistance in the offspring and a higher risk of adiposity, gestational diabetes and Type 2 Diabetes Mellitus (T2DM) in the affected mother. Folic acid and vitamin B12 are essential nutrients required by humans due to their involvement in cell formation and maintenance and participation in several vital reactions. Intracellularly, folic acid and vitamin B12 provide continuous critical support in maintaining the genomic stability of human cells through the prevention of chromosomal breakage, hypomethylation of DNA and myelin destabilization. Therefore, the aim of this study was to examine the underlying molecular mechanisms in human adipocytes affected by changes in folate and B12 concentrations using microarrays. One of the pathways significantly affected was the unfolded protein response (UPR) or endoplasmic reticulum (ER) stress pathway, which has recently been shown to be vital for initiation and integration of inflammation and insulin action in adipocytes. Therefore the effect of B12 and folate on this pathway and related pathogenesis in human adipocytes was investigated.

Materials and methods: Human pre-adipocyte cell line (Chub-S7) were grown and fully differentiated in different concentrations of vitamin B12 and folate; (1) Normal: Normal B12 (0.5 μ M) + Normal folate (6 μ M); (2) Null: No B12 + No folate; (3) F30: No B12 + High folate (30 μ M) and (4) F60: No B12 + High folate (60 μ M). The cells were harvested for RNA, conditioned media and protein after day 9 (d9) and 14 (d14) post-differentiation. Total RNA was isolated from Chubs-S7 (d9 and d14 post differentiation) and reverse transcribed to cDNA for microarray and quantitative real-time PCR analysis. Protein was used for western blotting.

Results: Microarray analysis revealed that ER stress was significantly upregulated in cells cultured in high folate conditions compared to the cells cultured in normal media. The two main pathways activated under these conditions were PERK and ATF6. Western blotting showed downstream target of PERK induced phospho-eukaryotic translation initiation factor 2- α kinase (p-eIF2 α) ($p < 0.05$) was significantly upregulated under high folate conditions. Furthermore, qRT-PCR showed that the downstream targets and other protein chaperones expressions were also increased in high folate conditions such as HERPUD1 ($p < 0.02$), activating transcription factor 4 (ATF4), eIF2 α and protein disulfide isomerase (PDI). mRNA expression levels of asparagine synthetase (ASNS): Normal vs F60, d14 ($p < 0.02$); Null vs F60 ($p < 0.01$), ERO1 α : Normal vs F60, d14 ($p < 0.04$), ubiquitin protein ligase E3B (UBE3B): Normal vs Null, d9 ($p < 0.05$) and activating transcription factor 6 (ATF6): Normal vs F60, d9 ($p < 0.02$) were also significantly altered.

Conclusion: Our results highlight that an imbalance of folate and B12 induces the expression of key genes mediating ER stress, promoting inflammation and inducing insulin resistance. Taken together these data suggest that a B12/Folate imbalance leads to a higher risk of developing obesity and T2DM. As a consequence, this study highlights that even subtle changes in the micronutrients (folate and B12) may have profound effects on the gene networks that regulate key metabolic functions.

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Early induction of 11 β -HSD1 expression in 3T3-L1 preadipocyte differentiation by cAMP increasing agents

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Background and aims: Adipose tissue production of active glucocorticoids from inactive metabolites is considered a pathogenic mechanism for metabolic syndrome and type 2 diabetes mellitus. Cortisone activation in tissues is mediated by 11 β Hydroxysteroid Dehydrogenase type 1 (11 β -HSD1) and Hexose 6 Phosphate Dehydrogenase (H6PDH) which co-operate in reducing cortisone to cortisol. 11 β -HSD1 protein is undetectable during the first stages of 3T3-L1 adipogenesis and is heavily upregulated in fully differentiated adipocytes. For this reason 11 β -HSD1 activity is considered part of the terminal differentiated adipocyte phenotype with no relationship to adipocyte differentiation regulation. Nevertheless adipose vascular stromal cells and 3T3-L1 preadipocytes, are able to differentiate in the presence of inactive corticoids, what requires 11 β -HSD1 activity during the first steps of differentiation. The aim of our work was to investigate, at the mRNA level, the gene expression regulation of the cellular system for glucocorticoid production during the first stages of adipose differentiation. In addition we explored individually the action of in vitro adipogenic inducers on 11 β -HSD1 mRNA level to identify in vivo candidates for the process.

Materials and methods: 3T3-L1 preadipocytes were differentiated by exposing confluent culture to a differentiation medium containing 10% Fetal Bovine Serum, glucocorticoid, insulin and 3-isobutyl 1-methylxanthine (IBMX) as an intracellular cAMP increasing agent. Total RNA was purified from the cells, cDNA was synthesized by a retrotranscriptase reaction and quantified by Real Time PCR.

Results: Quantification of 11 β -HSD1 mRNA by quantitative PCR during the first hours after differentiation induction shows a two fold increase at 8 hours and an exponential rising afterwards that reaches 2000 folds the level of expression in confluent 3T3-L1 preadipocytes before induction. This regulation seems to be specific for 11 β -HSD1 since H6PDH, which cooperates with 11 β -HSD1 in glucocorticoid reactivation, is not regulated during early differentiation. Separate incubation of confluent 3T3-L1 for 24h with the components of the differentiation cocktail clearly indicates that upregulation of 11 β depends on IBMX and suggests that glucocorticoids have an inhibitory effect. Time course experiments during 3T3-L1 differentiation indicate that early regulation of 11 β -HSD1 doesn't depend on upregulation of CCAAT/Enhancer-Binding Protein β or δ (CEBP- β , CEBP- δ) which are known regulators of 11 β -HSD1 promoter.

Conclusion: The ability of 11 β -HSD1 to respond to intracellular cAMP increase as an early differentiation event suggests that 11 β -HSD1 may be part of a preadipocyte stress response mechanism relevant for adipose tissue function.

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PS 055 Adipose tissue lipolysis and metabolism

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Bariatric surgery increases regional glucose uptake in adiposity

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Introduction: Studies have shown that bariatric surgery improves insulin sensitivity and decreases adipose tissue masses. The aim of this study was to investigate the effect of bariatric surgery on adipose tissue regional glucose uptake (GU).

Methods: The study consisted of 23 morbidly obese subjects (BMI = 42.6 ± 3.5 kg/m²), 15 with diabetes or pre-diabetes and 8 non-diabetics and they were compared with 10 healthy volunteers (BMI = 23.7 ± 0.9 kg/m²). All the obese subjects were randomized for two bariatric surgery techniques, either gastric sleeve (n = 10) or gastric bypass (n = 13). Abdominal GU was measured using positron emission tomography and ¹⁸F- fluoro-deoxy-glucose at fasting state and during euglycemic hyperinsulinemic clamp before and six months after the surgery. Magnetic resonance imaging (MRI) was used for abdominal fat mass assessment and anatomical reference.

Results: Bariatric surgery decreased average body weight by 26.9 ± 8.3 kgs (p<0.01) after six months. Abdominal subcutaneous adipose tissue (SAT) mass decreased postoperatively 7.5 ± 3.9 kg (p = 0.002) and 6.6 ± 1.3 kg (p < 0.001) respectively; and abdominal visceral adipose tissue (VAT) mass by 1.2 ± 0.5 kg (p = 0.001) and 1.8 ± 1.1 kg (p < 0.001) respectively. Whole-body insulin mediated glucose uptake (M value) was significantly impaired both in diabetics (11.8 ± 5.2 μ mol/min/kg) and in non-diabetics (14.0 ± 7.0 μ mol/min/kg) compared to controls (40.3 ± 9.5 μ mol/min/kg), both p < 0.001, and increased by 119 % (p < 0.001) in diabetics and by 81 % in non-diabetics (p = 0.001) six month after bariatric surgery. At fasting state, VAT and SAT GU remained unchanged after the surgery and no group differences were observed pre- or postoperatively with healthy controls. Insulin stimulated VAT and SAT GU increased postoperatively in all patients (by 3.4 ± 1.4 μ mol/min/kg, p=0.015 and by 3.7 ± 0.9 μ mol/min/kg, p=0.001 respectively). However, no differences in metabolic effects were observed between diabetic and non-diabetic obese patients.

Conclusion: Weight loss through bariatric surgery improved insulin stimulated regional glucose uptake in both abdominal visceral and subcutaneous adipose tissue in which sleeve and gastric bypass techniques effect similarly in diabetic and non-diabetic obese.

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Abdominal adipocyte size predicts liver fat independent of age, gender, BMI, body fat distribution and PNPLA3 genotype in nondiabetic subjects

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Background and aims: Adipocyte hypertrophy has been suggested to be causally linked with ectopic lipid accumulation in the liver. Factors known to regulate liver fat in humans include body mass index (BMI), body fat distribution and genetic variation in the patatin-like phospholipase domain-containing 3 gene (PNPLA3; adiponutrin) at rs738409 (16 positive studies). It is unknown, however, whether adipocyte size predicts liver fat content independently of these factors and, if so, how much of the variation it explains.

Materials and methods: Liver fat (¹H-MRS), intra-abdominal (IA) and abdominal subcutaneous adipose tissue (SC) volume (MRI), abdominal adipocyte size (collagenase digestion, light microscope) and the PNPLA3 genotype were determined in 113 nondiabetic subjects (age 40 ± 14 years, BMI 30.0 ± 5.5 kg/m², male-to-female ratio 55/78). Multiple linear regression analysis was used to identify independent predictors of liver fat content.

Results: In multiple linear regression, age, gender, BMI, the IA/SC ratio and PNPLA3 genotype explained 43% of variation in liver fat content. Addition

of adipocyte size into the model increased the percent of explanation to 52%. Independent predictors included age ($p=0.011$), BMI ($p<0.001$), PNPLA3 genotype ($p<0.001$), the IA/SC ratio ($p<0.001$) and adipocyte size ($p<0.001$) (Table 1).

Conclusion: Adipocyte size is a highly significant independent predictor of liver fat. While age, gender, BMI and the IA/SC ratio explain 43% of the variation in liver fat, addition of adipocyte size increases it to 52%.

	Unstandardized coefficients ± SD	Standardized coefficients Beta	Significance	Unstandardized coefficients ± SD	Standardized coefficients Beta	Significance
Model 1 ($R^2=43\%$)				Model 2 ($R^2=52\%$)		
(constant)	-0.448±0.471		0.343	(constant)	-1.385±0.479	0.005
Age	-0.006±0.004	-0.141	0.142	Age	-0.010±0.004	0.011
Gender	-0.182±0.122	-0.143	0.139	Gender	-0.167±0.112	0.131
BMI	0.053±0.008	0.492	<0.001	BMI	0.032±0.009	0.296
log(IA/SC)	0.885±0.204	0.463	<0.001	log(IA/SC)	0.852±0.188	0.446
PNPLA3	0.189±0.065	0.214	0.004	PNPLA3	0.236±0.061	0.266
				Adipocyte size	0.015±0.003	0.397
						<0.001

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Expression of anti-lipolytic receptors in human adipose tissues

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Background and aims: Anti-lipolytic signals in adipose tissue could be related to insulin sensitivity through the generation of systemic non-esterified fatty acids. Recently uncovered G-coupled proteins receptors GPR109A, GPR109B and GPR81 respond to ligands produced in intermediary metabolism and convey an anti-lipolytic signal in adipose tissue. The endogenous ligand for GPR109A is beta-OH butyrate whereas lactate is the endogenous ligand for GPR81. Little is known about the regulation of these receptors in human adipose tissue. We hypothesize that depot-specific expression of these receptors could help explain tissue anti-lipolytic effects.

Materials and methods: Due to the high degree of sequence similarity between GPR109A and GPR109B, the existing commercially available Taqman gene expression assays were tested for specificity on in-house developed specific templates for the two genes. Both gene assays were found to be non-specific. Following this, specific expression assays were developed and validated. Expression for all three genes in human adipose tissue was determined using paired abdominal and gluteal biopsy samples from 20 healthy male and 20 healthy female subjects. The mRNA expression assays were run on an ABI-7900HT system and normalized to PPIA and PGK1. To assess the relevance of possible feedback loops, the plasma concentrations of the endogenous ligands were measured in plasma.

Results: GPR81 abdominal subcutaneous adipose tissue expression correlated positively with GPR109A ($p=0.0001$, $r=0.56$) and GPR109B ($p=0.001$, $r=0.49$) expression in the same depot, whereas the expressions of GPR109A and GPR109B were unrelated to each other. GPR109A showed a 1.34 fold higher expression in gluteal tissue compared to abdominal tissue ($p=0.012$). GPR109B showed a 1.45 fold increase in expression in gluteal tissue compared with abdominal tissue ($p=0.006$). No depot difference was observed for GPR81. Gender differences were not detected amongst any of the genes. When looking for effects of obesity, abdominal expression of GPR81 was 1.17 fold lower in obese individuals ($p=0.013$, $n=20$) driven by an 1.26 fold reduction of expression in the male group ($p=0.041$, $n=10$). No obesity effects were seen for the other genes. No correlations were seen between measured levels of beta-OH butyrate and the expression of its receptor GPR109A. The gluteal expression of GPR81 was negatively correlated with lactate levels overall ($p=0.026$, $r=-0.33$, $n=40$) and more so in obese men ($p=0.006$, $r=-0.79$, $n=10$).

Conclusion: GPR109A and GPR109B showed adipose tissue depot specific expression differences. Higher expression of the anti-lipolytic GPR109A was seen in the gluteal tissue, known for its lower lipolysis rate. Obesity did not affect the expression of GPR109A and GPR109B but lowered the expression of GPR81. Furthermore, plasma lactate (the ligand to GPR81) was negatively associated to GPR81 gene expression which suggests a ligand-receptor auto-feedback.

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Is BrCa1 behind decreased lipogenic gene expression in adipose tissue from obese subjects?

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Background and aims: Gene expression of the main lipogenic enzymes is paradoxically decreased in obesity, but the mechanisms behind these findings are poorly known. Breast Cancer 1 (BrCa1) has been described to interact with acetyl-CoA carboxylase (ACC) reducing the rate of fatty acid biosynthesis in lipogenic tissues.

Materials and methods: BrCa1 gene expression, total and phosphorylated (P-)BrCa1 and ACC were analyzed in adipose tissue samples obtained from a total sample of 133 subjects. BrCa1 gene expression was also analyzed during in vitro differentiation of human adipocytes and 3T3-L1 cells.

Results: BrCa1 gene expression was significantly up-regulated in both omental (OM; 1.36-fold, $p=0.002$) and subcutaneous (SC; 1.49-fold, $p=0.001$) adipose tissue from obese subjects. In parallel, P-ACC was increased in SC ($p=0.007$) as well as in OM fat ($p=0.010$). Consistent with its role of limiting fatty acid biosynthesis, BrCa1 mRNA was up-regulated in pre-adipocytes (both mRNA, 3.5-fold, $p<0.0001$, and protein, 1.2-fold, $p=0.001$) and decreased during adipogenesis in both human adipocytes and 3T3-L1 cells, while P-ACC decreased during differentiation of adipocytes ($p=0.005$) allowing lipid biosynthesis.

Conclusion: The specular findings of BrCa1 and lipogenic enzymes in adipose tissue and during differentiation of adipocytes suggest that BrCa1 might help to control fatty acid biosynthesis in adipocytes and adipose tissue from obese subjects.

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Effects of dexamethasone on gene expression and glucose uptake in human subcutaneous and omental adipose tissue

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Background and aims: Endogenous cortisol production is suggested to play a role in the development of the metabolic syndrome, visceral obesity and T2DM. We studied the effect of the synthetic glucocorticoid dexamethasone (Dex) on gene expression and glucose uptake capacity in human subcutaneous and omental adipose tissue aiming to identify new mechanisms and biomarkers for insulin resistance.

Materials and methods: Subcutaneous and omental adipose tissue were obtained from 17 patients going through elective abdominal surgery for non-malignant disorders (8 M/9 F; age 28-57 yrs; BMI 20.7-30.6 kg/m²). The adipose tissue was incubated in absence or presence of Dex (0.01-3 μM) for 24 h. After incubation, adipose tissue was saved for RNA extraction and adipocytes were isolated for glucose uptake analysis and cell size determination. DNA microarray analysis (Affimetrix, Human Exon 1.0 ST

Array) was performed in a pilot subgroup of 4 males and pathway analysis was performed.

Results: Dex changed the expression of 527 genes in both subcutaneous and omental adipose tissue at a maximally effective concentration (3 μ M). Pathway analysis of Dex-regulated genes showed a clear over-representation of functions and pathways related to inflammation. Single genes affecting lipolysis, glucose uptake and oxidation or adipocyte differentiation was changed after Dex incubation. Only two genes were differently regulated by Dex between the depots; CYP4Z1 (cytochrome P450, family 4, subfamily Z, polypeptide 1) expression increased in subcutaneous, but not in omental adipose tissue, and C13orf36 (chromosome 13 open reading frame 36) expression decreased in omental but not in subcutaneous adipose tissue. The expression of the secreted peptides leptin and TIMP4 (metallopeptidase inhibitor 4) were increased by Dex in both depots. Dex (3 μ M) exerted a greater inhibition of basal glucose uptake in omental vs subcutaneous adipocytes (by about 40%, $p < 0.01$ vs 30%, $p < 0.05$; $n = 10$). Dex (0.01–3 μ M) had a concentration dependent effect on basal and maximal insulin stimulated glucose uptake in both subcutaneous and omental adipocytes ($n = 4$). EC50 for the Dex effect to inhibit glucose uptake was about 2-fold lower in omental compared to subcutaneous adipocytes. Adipocyte size was not influenced by Dex in either of the adipose tissue depots.

Conclusion: Dex at maximally effective concentration similarly influences gene expression in both subcutaneous and omental adipose tissue depots. Adipocyte glucose uptake capacity is reduced by Dex, with omental adipocytes tending to be more sensitive than subcutaneous adipocytes. Leptin and TIMP4 are potential candidates to be used as circulating biomarkers for glucocorticoid-mediated insulin resistance. The gene expression changes are currently being evaluated with respect to magnitudes, dose-response relationships and links to glucose metabolism in the two fat depots.

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Inverse regulation of lipolysis in different intra-abdominal fat depots in mice

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Background and aims: Given the strong link between visceral adiposity and morbidity including hepatic steatohepatitis, it is crucial to characterize obesity-associated alterations in adipocyte function particularly in fat depots drained to the liver. Yet, such depots are not easily accessible in humans, and the most frequently-studied depot in rodent models is the perigonadal fat pad, which is drained systemically. Thus, here we aimed to study metabolic alterations in mesenteric compared to perigonadal adipocytes in mice.

Materials and methods: C57BL/6J mice were put on chow or high fat diet (HFD) for 8 weeks. Adipocytes from perigonadal and mesenteric fat depots were isolated by collagenase digestion. Viability and lipolysis (basal, isoproterenol- and insulin-stimulated) were measured and size of adipocytes was determined. Protein levels and mRNA expression were determined by Western blot technique and real-time RT-PCR, respectively.

Results: Relative increase in adipocyte size due to HFD was higher in perigonadal compared to mesenteric adipocytes (55.6 \pm 5.6% in perigonadal vs. 37.6 \pm 3.4% in mesenteric, $p < 0.05$). In chow-fed mice, the anti-lipolytic effect of insulin was higher in perigonadal compared to mesenteric adipocytes (42.4 \pm 4.6% in perigonadal vs. 10.2 \pm 3.5% in mesenteric, $p < 0.01$), while there was no difference under HFD (9.6 \pm 9.2% in perigonadal vs. 25.3 \pm 12.9% in mesenteric, $p = 0.2$). Basal NEFA release in perigonadal adipocytes was significantly higher in HFD- compared to chow-fed mice (4.5 \pm 0.5 mmol/10⁶cells*60min in chow-fed vs. 8.5 \pm 1.2 mmol/10⁶cells*60min in HFD-fed, $p < 0.01$) whereas there was no increased lipolysis in mesenteric adipocytes upon HFD (25.1 \pm 9.3 mmol/10⁶cells*60min in chow-fed vs. 13.3 \pm 2.0 mmol/10⁶cells*60min in HFD-fed, $p = 0.2$). Consistently, while high fat feeding led to an approx. 45% increase of NEFA levels in systemic circulation ($p = 0.05$), NEFA levels in portal blood were comparable between chow- and HFD-fed mice. In addition, protein levels of the G_o/G_i switch gene 2 (GOS2), which were previously found to be inversely related to basal lipolysis, decreased under HFD in perigonadal adipocytes but increased in mesenteric adipocytes. Similarly, perilipin levels (mRNA and protein) were differently regulated between the two depots.

Conclusion: Lipolysis is differently regulated between perigonadal and mesenteric adipocytes and these depot-specific differences might be explained by altered regulation of GOS2 and/or perilipin content.

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Characterisation of the metabolic fluxes in the adipocyte differentiation process using bioinformatics tools

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Background and aims: White adipose tissue mass is a critical factor determinant for obesity and associated health risks. The formation of new adipocytes from the differentiation of pre-adipocytes is accompanied with important metabolic changes. Stable isotope tracer data is used to reveal the metabolic flux profile in cells under studied conditions, and thus to provide an insight into the cell phenotype. The *de novo* adipocyte differentiation and the associated changes of metabolic flux profile were studied on the 3T3-L1 cell line incubated with stable isotope tracers. The use of a software simulating the measured data ensures the acquisition of more information and the elaboration of a metabolic map, taking into account all the available information throughout the differentiation process.

Materials and methods: 3T3-L1 preadipocytes were cultured with 25 mM glucose DMEM medium with 10% NCS and differentiated with DMEM containing 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μ M dexamethasone and 1 μ M insulin (designated day 0). At day 3 the medium was replaced by DMEM containing 10% FBS and insulin, and at day 6 the medium was changed to DMEM with 10% FBS, and was changed every two days. Biochemical determinations of glucose, lactate, glutamate and glutamine were performed from day 0 to day 9. Incubations with 25 mM glucose 50% enriched with [1,2-¹³C₂]-D-glucose were performed for 24 hours at day 0 (preadipocytes), day 4 (immature adipocytes) and day 8 (mature adipocytes). The biochemical concentrations and isotopologic patterns of glucose, lactate, glutamate, glycogen, ribose and fatty acids were measured at the beginning and at the end of the incubations, the rates of production and consumption were calculated, and Mass Isotopomer Distribution Analysis and flux estimation with our software Isodyn was performed.

Results: The biochemical measurements indicated three distinct periods. Day 0 to day 4 was characterized by a glucose-to-lactate ratio of 1:2 and positive glutamine consumption. Days 4 to 7 the glucose consumption increased, although the glucose-to-lactate ratio approached 1:1, and from day 7 the consumption of glucose decreased, and glutamine production was observed. Using the isotopologue data obtained in our experiments and our custom metabolic software Isodyn we quantified the metabolic flux distribution in the glucose metabolic network.

Conclusion: The analysis of the isotopic distribution allowed us to evaluate the dynamics of metabolic fluxes in the central carbon metabolism. The results are consistent with previous works in the field. Dramatic changes in glycogen accumulation were observed throughout the differentiation process, and a metabolic rearrange in anaplerotic pathways was observed. The analysis with Isodyn allows us to define the whole set of metabolic fluxes inside the cell, defining the changes in the metabolic phenotype during the differentiation process.

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Low B₁₂ and high folate: a novel regulator of cholesterol biosynthesis in human adipocytes

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Background and aims: Vitamin B12 and folate play a crucial role in genomic stability of human cells and several other critical pathways during development and adult life. Peri-conceptional nutrition is associated with increased metabolic risk in the offspring. Prospective, longitudinal studies in humans show low maternal vitamin B12 and high folate levels independently predict: higher metabolic risk & insulin resistance in the offspring, higher risk of adi-

posity, gestational diabetes and type 2 diabetes in the affected mother. This is supported by an animal study, which showed that B12 and folate mediate a vital role in determining offspring's metabolic risk by DNA methylation. "Low B12 and high folate" imbalance is increasingly common, may partly be due to folic acid fortification programmes. It is intriguing that such "imbalance" causes hyperhomocysteinaemia, higher incidence of anaemia & cognitive dysfunction in elderly and higher metabolic risk of offspring when present in mothers. Therefore, to elucidate the underlying molecular mechanisms, gene expression analysis was done to study the effect of B12 and folate on human pre-adipocyte cell line using microarray analysis.

Materials and methods: Human pre-adipocyte cell line Chub-S7 was grown and fully differentiated in different concentrations of vitamin B12 and folate such as (1) Normal: Normal B12 (0.5µM) + Normal folate (6µM); (2) Null: No B12 + No folate; (3) F30: No B12 + High folate (30µM) (4) F60: No B12 + High folate (60µM) for 14–21 days. Total RNA was then isolated for microarray and reverse transcribed for quantitative real-time PCR analysis.

Results: Microarray analysis revealed that 491 genes were differentially expressed significantly ($p < 0.05$) in adipocytes treated with different concentrations of B12 and folate compared to normal B12/folate levels. Our results screened that genes involved in different biological processes and metabolic pathways were modulated including the cholesterol synthesis pathway. Differential array expression analysis examined that genes involved in biosynthesis of cholesterol occurring in three important phases: (1) mevalonate formation from acetyl-CoA by 3-hydroxy-3-methyl glutaryl-CoA synthetase (HMGCS1) & 3-hydroxy-3-methyl glutaryl-CoA reductase (HMGCR) (2) conversion of mevalonate to squalene by isopentenyl diphosphate isomerase (IDI1) and (3) squalene conversion to cholesterol by sterol C4 methyl-oxidase-like (SC4MOL) & squalene epoxidase (SQLE) were significantly modulated ($p < 0.001$ to $p < 0.05$). In addition, genes regulating cholesterol levels such as insulin induced gene-1 (INSIG1) ($p < 0.01$) and StAR-related lipid transfer domain4 (STARD4) were significantly up-regulated ($p < 0.05$). Validation by real-time PCR confirmed that these genes ($p < 0.01$ to $p < 0.05$) were up-regulated by different concentrations of B12 and folate compared to normal B12/folate levels.

Conclusion: Our data provides novel evidence that imbalance of B12/folate alters gene expression in cholesterol biosynthesis and regulation, thus potentially predisposing to obesity-associated dyslipidemia and insulin-resistance syndromes. This is the first report, unravelling the powerful effect of these micronutrients on gene networks that regulate many important metabolic functions in human adipocytes.

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PS 056 Adipose tissue inflammation

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Autophagy activity is up regulated in adipose tissue of obese individuals and controls pro-inflammatory cytokine expression

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Background and aims: Autophagy is a cellular mechanism which rearranges the cellular structure and provides essential nutrients by degrading intracellular organelles or protein aggregates. Essential autophagy-related proteins are LC3 and ATG7. Recent studies have shown that autophagy also governs pro-inflammatory cytokine production in peripheral blood mononuclear cells. The development of obesity is often accompanied by a chronic low grade inflammation in adipose tissue. Together, these results suggest that autophagy may regulate inflammation in adipose tissue. We hypothesized that altering autophagy activity will affect pro-inflammatory cytokine expression in adipocytes.

Materials and methods: Human and mouse adipose tissue and human SGBS adipocytes were treated with the autophagy inhibitor 3-methyladenine (10 mM) (3MA) or siRNA to block ATG7. Expression and production of cytokines was evaluated by Real-time PCR, ELISA and western blotting techniques.

Results: Our results show that autophagic activity is increased in adipose tissue of obese animals (1.44 ± 0.12 LC3:actin ratio in wt mice vs. 12.32 ± 1.188 in obese mice; $p < 0.001$). Similarly, levels of the autophagy marker LC3 were elevated in subcutaneous adipose tissue of obese vs. lean individuals (0.24 ± 0.031 LC3:actin ratio in lean vs. 0.51 ± 0.13 in obese individuals; $p < 0.05$). Treatment of human subcutaneous adipose tissue explants with 3MA to reduce autophagy led to a significant increase in IL-1 β , IL-6 and IL-8 gene expression (relative fold change of 1,00 vs. 6.75 ± 0.68 for IL-1 β , 1,00 vs. 22.53 ± 17.10 for IL-6 and 1,00 vs. 6.90 ± 1.50 for IL-8; $p < 0.05$). In addition, secretion levels of the pro-inflammatory cytokines by subcutaneous adipose tissue explants were similarly enhanced after treatment with 3MA (1318 ± 244.0 vs. 3782 ± 618.2 pg/ml/g IL-1 β , 326.9 ± 78.93 vs. 643.6 ± 84.25 ng/ml/g IL-6 and 514.6 ± 167.4 vs. 1480.0 ± 160.4 ng/ml/g IL-8; $p < 0.05$). Treatment of visceral adipose tissue explants and SGBS adipocytes with 3MA showed comparable results. Alternatively, blocking of the autophagy protein ATG7 activity in vitro using small interfering RNA (siRNA) treatment led to similar results since it promoted inflammatory gene expression in SGBS cells (relative fold change of 1,00 vs. 3.93 ± 0.30 for IL-1 β , 1,00 vs. 3.79 ± 0.36 for IL-6 and 1,00 vs. 15.90 ± 2.52 for IL-8; $p < 0.05$).

Conclusion: Our results demonstrate that autophagy activity is up regulated in adipose tissue of obese individuals and controls pro-inflammatory gene expression. Autophagy may function to regulate inflammatory gene expression and prevent chronic inflammation in adipose tissue.

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Transcriptomic analysis of macrophage polarisation: role of PPAR γ in alternative activation

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Background and aims: Human macrophages (HM) undergo different forms of activation in response to cytokines. Classical activation (M1) displays pro-inflammatory properties, and can be induced by IFN γ and/or LPS. Alternative activation (M2a) shows immuno-regulatory properties and is promoted by IL-4, whereas IL-10 produces a "deactivated" state, with immunosuppressing characteristics (M2c). Glitazones are the newest class of oral type-II antidiabetic agent and are a selective agonist for peroxisome proliferator activated receptor gamma (PPAR- γ). The PPAR γ system appears to be implicated in M2a activation enhancing their anti-inflammatory properties. The aim of the study is the evaluation of the biological processes involved in the different types of HM activation compared to the effects induced by exposure to PPAR γ -agonists.

Materials and methods: HM were stimulated with INF γ +LPS (M1), IL-4 (M2a), IL-10 (M2c), or a PPAR γ -agonist (GW1929-Sigma). Phenotypic changes for each experimental condition were investigated through whole-genome transcriptomic analysis by microarrays (Agilent-Technologies).

Gene ontology (GO) analysis was performed using Gorilla tool. Validation was obtained by RT-PCR on the most differentially expressed genes.

Results: After characterization of M1, M2a, and M2c genomic profiles, we investigated the differences between PPAR γ -stimulated HM and M1, observing a significant decrease in immunitary and inflammatory processes (corresponding GO-term's p -value=2.00*10⁻³⁵ and 1.20*10⁻²⁰) and a great increase in cell cycle activity (p =1.51*10⁻³⁵). Similar, but quantitatively lower, results were observed also in comparison with M2a and M2c, although these two subpopulations are usually regarded as anti-inflammatory.

Conclusion: Exposure of HM to a PPAR γ -agonist induces a transcriptomic phenotype closer to M2a/M2c than to M1. Nevertheless, PPAR γ -agonist activation decreases inflammatory cytokine production and increases cell-cycle gene expression also compared to cytokine-induced M2a and M2c activation, suggesting a specific role of PPAR γ in the regulation of macrophage differentiation. These results can partially explain the anti-inflammatory properties of glitazones, actually widely employed in clinical practice.

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Physical exercise improves infiltration and polarisation of macrophage in white adipose tissue of diet-induced obesity rats

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Background and aims: Increased infiltration of white adipose tissue (WAT) by macrophages plays an important role in the establishment of the chronic inflammatory state and metabolic dysfunction (e.g., insulin resistance) that is associated with obesity. In obese animals, macrophages assume a proinflammatory classical activation profile also known as M1, in contrast to lean conditions with an alternative activation state (M2) and production of immunosuppressive factors, such as IL-10. Although physical exercise might decrease proinflammatory status in WAT, it remains uncertain whether exercise affects the adipocytes or the infiltrated macrophages. The purpose of the current study was to analyze the effects of acute and chronic swimming exercises on the inflammatory status and insulin signaling of the adipose tissue fractions, stromal-vascular fraction (SVF) and adipocytes.

Materials and methods: The effects of acute and chronic exercise protocols were investigated on circulating IL-6 and TNF- α levels by an enzyme-linked immunosorbent assay, insulin signaling proteins and cytokine expression by Western Blot analysis and mRNA cytokine levels by Real Time in WAT fractions of diet-induced obesity (DIO). Additionally, we also observed macrophage infiltration and polarization in WAT through immunohistochemistry.

Results: The results showed that acute exercise reduced the proinflammatory status mainly in SVF evidenced by reduced expression of MCP-1, TNF- α and IL-1 β , without changing WAT macrophage infiltration. In this line, the chronic exercise displayed the same results and also completely reversed the macrophage infiltration into WAT. In parallel, both exercise protocols increase the IL-10 protein content and also promote reduction in circulating TNF- α levels. We also observed higher insulin-induced phosphorylation of IR, IRS-1 and Akt in both fractions in all exercised animals. Our results show that physical exercise in DIO rats induces not only an important suppression in proinflammatory cytokines, but also increases anti-inflammatory cytokines secretion.

Conclusion: Taken together these alterations indicate that acute and chronic exercises contribute to improved inflammatory status and insulin signaling in WAT by different mechanisms. Acute exercise induces only the phenotypic switching from M1 to M2 macrophages. On the other hand, besides improving the polarization, the chronic exercise blunted macrophage infiltration induced by obesity. These data provide considerable progress in our understanding of the molecular events that link physical exercise to an improvement in inflammation and insulin resistance in WAT.

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Foam cells formation in human visceral adipose tissues correlates with increase in BMI

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Background and aims: Inflammation is one of the mechanisms proposed to link obesity with its associated morbidities, including type 2 diabetes and

cardiovascular diseases. Macrophages accumulate in adipose tissues in proportion to obesity and adipose tissue macrophages (ATMs) suggested to promote insulin resistance and type 2 diabetes. We aimed to study the molecular characteristics of human lipid-laden ATMs and correlate it with the clinical parameters associated with the metabolic syndrome.

Materials and methods: Histological sections of human fat biopsies (visceral and sub-cutaneous adipose tissues) were stained with CD68 and hematoxylin-eosin to identify macrophages and lipid-laden ATMs, respectively. Using flow cytometry, we developed a technique to detect and quantify lipid-laden ATMs by gating on CD45⁺, CD14⁺ cells and analyze their lipid profile by staining with Bodipy for neutral lipids.

Results: We found in human fat biopsies a sub-group of macrophages loaded with lipids, which we named adipose tissue (AT) foam cells, as they resemble foam cells found in atherosclerotic lesions. AT foam cells accumulated in crown-like structures areas surrounding the adipocytes. Foam cells were detected in fat biopsies of obese and diabetic but not lean patients and were more evident in visceral comparing to subcutaneous adipose tissues. In diabetic patients AT foam cells were associated with matrix deposition in crown-like structures. Based on flow cytometry analyses, we found that formation of AT foam cells correlated with increase in the BMI of the patients. For example, for BMI 20.1: 0.05% AT foam cells, for BMI 40: 12.4% AT foam cells, for BMI 43.7: 24.4% AT foam cells and for BMI 50, 53.7% AT foam cells were found.

Conclusion: We discovered in adipose tissues of obese, diabetic but not lean patients formation of AT foam. Higher frequency of AT foam cells correlated with increase in the BMI and in visceral adiposity of the patients. We plan to further study the molecular characteristics of AT foam cells and correlate them with other clinical parameters associated with obesity and its related morbidities. Together, using these approaches, we will be able to determine whether foam cells generation in visceral adipose tissues of obese patients contribute to adipose tissues insulin resistance and promoting the systemic insulin resistance and type 2 diabetes.

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Dietary fatty acids activate TLR4 and the inflammasome in dendritic cells and adipocytes - implications for diet-induced obesity

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Background and aims: Adipose tissue inflammation has been linked with a plethora of pro-inflammatory cytokines which are thought to play a key role in the attenuation of insulin sensitivity. Several recent studies suggest a functional role for the NLRP3 inflammasome, a protein complex involved in processing IL-1 and IL-18, in obesity, with specific roles in adipogenesis and development of adipocyte insulin resistance (IR). However the critical metabolic trigger of inflammasome activation within the context of diabetes remains elusive.

Materials and methods: C57BL/6J mice received a HFD for 0-16 weeks. Glucose tolerance test (GTT) determined IR. Plasma, adipose and bone marrow (BM) were harvested. Stromal vascular fraction (SVF) cells were isolated from adipose and analyzed by flow cytometry for CD11c⁺CD11b⁺F4/80⁺ DC. BM cells were cultured in RPMI supplemented with GM-CSF for 7 days. DC were stimulated with LPS (100ng/ml) and harvested for protein and RNA. BMDC were primed for 3h with either LPS (100ng/ml) or PA (200 μ M) to induce pro-IL-1 β production followed by 1h ATP stimulation (5mM), a well characterised activator of the inflammasome.

Results: We demonstrate that dietary fatty acids directly prime and activate the NLRP3 inflammasome via TLR4, promote recruitment/activation of adipose DC (CD11c⁺CD11b⁺F4/80⁺) in high-fat diet (HFD) induced IR. HFD-derived BMDC exhibited greater LPS responsiveness with increased IL-1 β secretion, enhanced IL-1R1, TLR4 and caspase-1 expression. Adipocyte/HFD-derived BMDC co-culture enhanced adipocyte IL-1 β secretion, caspase-1 activity, with greater adipocyte IR, versus age-matched chow fed controls. Complimentary *in vitro* experiments demonstrate saturated fatty acid (SFA) treatment (palmitic acid; PA) enhanced cytokine expression, which was attenuated by caspase-1 and TLR4 inhibition in BMDC. Pre-priming of BMDC with PA led to a dramatic rise in IL-1 β concentrations following exposure to ATP, a potent inflammasome activator.

Conclusion: SFA represent potential metabolic triggers priming the inflammasome, promoting adipocyte inflammation and IR, suggesting a direct effect of SFA on inflammasome activation in diet-induced obesity.

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The impact of hyperinsulinaemia on the serum IL-12/p40 subunit concentrationA. Nikolajuk^{1,2}, A. Adamska¹, M. Karczewska-Kupczewska¹, N. Kaminska¹, M. Zielinska¹, M. Górski¹, I. Kowalska¹, M. Strączkowski¹;¹Endocrinology, Diabetology and Internal Medicine, Medical University of Białystok, ²Department of Prophylaxis of Metabolic Diseases, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Białystok, Poland.

Background and aims: Numerous studies indicate an association between low-grade chronic inflammation and predisposition to type 2 diabetes and atherosclerosis. IL-12 is a proinflammatory cytokine with proatherogenic properties. IL-12 is a disulfide-linked, 70kDa (p70) heterodimeric glycoprotein composed of a 40kDa (p40) subunit and a 35kDa (p35) subunit. Many data reported higher levels of p40 subunit than total IL-12. The aim of the present study was to investigate the influence of hyperinsulinemia on serum p40 subunit.

Materials and methods: Our study involved 35 young (age: 24.31±2.81 years), apparently healthy men with normal glucose tolerance. Anthropometric measurements, blood biochemical analysis and euglycemic hyperinsulinemic clamp were performed in the studied group.

Results: The serum concentrations of p40 was significantly lower after the clamp than the baseline state ($p<0.05$). The change in IL-12/p40 during the clamp was already to the steady-state insulin (SSI) concentrations ($r=0.35, p=0.037$) - the higher SSI the greater decrease in serum IL-12/p40. We found inverse correlations between post-clamp serum p40 and total cholesterol and LDL-cholesterol ($r=-0.34, p=0.049$ and $r=-0.46, p=0.006$, respectively). A significant association between basal and post-clamp p40 subunit and lymphocyte cell count ($r=0.35, p=0.037$ and $r=0.45, p=0.006$, respectively) and significant negative correlations with neutrophil cell count ($r=-0.41, p=0.014$ and $r=-0.51, p=0.002$, respectively) was observed in the studied group.

Conclusion: Our data indicated that hyperinsulinemia decreased serum IL12/p40 concentration.

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The expression of interleukin-33 and its receptor ST2 are increased in obese adipose tissue and predominantly found in endothelial cellsT.M. Stulnig¹, B. Wernly¹, S. Demyanets², C. Kaun², M. Hammerle³, B. Hantusch³, A. Neuhofer³, M. Keck³, O. Azsmann³, G. Prager³, J. Wojta², M. Zeyda¹;¹Clinical Division of Endocrinology and Metabolism, ²Clinical Division of Cardiology ³Medical University of Vienna, Austria.

Background and aims: Obesity is associated with a chronic inflammation of the adipose tissue, which contributes to obesity-associated complications such as insulin resistance and Type 2 diabetes. Interleukin(IL)-33, a novel member of the IL-1 family, acts via its receptor ST2 and is involved in the pathogenesis of inflammatory disorders including atherosclerosis and heart disease. IL-33 has been demonstrated to promote endothelial cell inflammatory response but also Th2 and M2 polarization of T cells and macrophages, respectively. IL-33 and its receptor ST2 have been shown to be expressed in human and murine adipose tissue, but alterations in obesity as well as a possible role in adipose tissue inflammation has not been investigated yet.

Materials and methods: We utilized murine obesity models as well as obese and lean individuals to investigate the impact of obesity on IL-33 and ST2 gene and protein expression levels in adipose tissue and blood and their correlation with obesity-associated parameters. By fractionation of adipose tissue we examined the cellular sources and location of IL-33 and ST2.

Results: We show that IL-33 and ST2 are markedly elevated in obese visceral and subcutaneous adipose tissue of humans and in diet-induced obese mice, but not in leptin receptor-deficient obese mice. Also soluble ST2, but not IL-33 plasma levels were elevated in obesity. As the main source for IL-33 within the adipose tissue, we identified endothelial cells. In human adipose tissue ST2 was almost exclusively found on endothelial cells, indicating these cells as the main target of IL-33 action. In contrast, T-cells were the predominantly ST2-expressing cell in mice. In human omental adipose tissue of obese IL-33 expression strongly correlated with leptin expression, while no other significant correlation with inflammatory, metabolic, and anthropometric data was detectable.

Conclusion: IL-33 expression in adipose tissue is regulated by obesity and endothelial cells are the main cell type involved in expression and, in humans, also action of IL-33. Thus, the adipose tissue microvasculature could participate in obesity-associated inflammation and related complications via IL-33/ST2.

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Implication of the inflammatory kinase Tpl2 in the deleterious effects of cytokines and macrophages on adipocytes functionsJ.-F. Tanti^{1,2}, F. Ceppo^{1,2}, T. Grémeaux^{1,2}, J. Jager^{1,2}, O. Bezy³, Y. Le Marchand-Brustel^{1,2}, M. Cormont^{1,2};¹Team Cellular and Molecular Pathophysiology of Obesity, INSERM U 895, ²Université de Nice Sophia Antipolis, Nice, France, ³Joslin Diabetes Center, Boston, USA.

Background and aims: Adipose tissue (AT) inflammation and dysfunction are involved in the development of the complications of obesity. In obesity, AT is infiltrated by inflammatory macrophages that contribute to the production of cytokines. These cytokines alter the functions of adipocytes leading to a decrease in insulin signaling and action and to an increase in lipolysis. Further, free fatty acids produced by dysfunctional adipocytes have an inflammatory effect on macrophages. This cross-talk may sustain the inflammation of the adipose tissue. We have recently reported that the MAP3 kinase Tpl2 was a new inflammatory kinase involved in the deleterious effect of cytokines on adipocytes function. The aim of the present study was to determine the implication of Tpl2 in the cross-talk between macrophages and adipocytes and in the deleterious effect of macrophages on adipocytes function.

Materials and methods: The expression of Tpl2 in AT macrophages was measured by real-time PCR after FACS sorting. Adipocytes were cultured with macrophages or incubated with conditioned medium from macrophages treated with a concentration of LPS that is compatible with the concentration found in obese mice or patients. All these treatment was performed in the absence or presence of a Tpl2 inhibitor in order to assess the implication of Tpl2 pathway on cytokines production, lipolysis, insulin signaling and action.

Results: We found that Tpl2 expression was increased in AT of obese mice and patients and that treatment of adipocytes with TNF-alpha or IL-1beta increased its expression in adipocytes. Further by FACS sorting, we demonstrated that Tpl2 expression was increased in CD11c+ macrophages from AT of obese mice. A co-culture between adipocytes and macrophages enhanced the expression and production of inflammatory cytokines and the lipolysis and decreased insulin signaling in adipocytes. We found that the pharmacological inhibition of Tpl2 in the co-culture markedly suppressed the production of cytokines and free fatty acids and partly restored the insulin signaling in adipocytes. A conditioned medium of macrophages treated with low dose of LPS inhibited insulin signaling and glucose transport in adipocytes. Treatment of macrophages with a Tpl2 inhibitor markedly reduced the deleterious effect of the conditioned medium on insulin signaling and action in adipocytes.

Conclusion: Our results indicate that the Tpl2 pathway may constitute a mediator in the cross-talk between adipocytes and macrophages in adipose tissue leading to sustained inflammation. Inhibition of this pathway may reduce the production of inflammatory cytokines by the macrophages and improved adipocytes insulin signaling and functions.

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The effect of age versus high-fat feeding on adipose tissue macrophage accumulation and immunogenicity

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Background and aims: High-fat diet (HFD)-induced obesity is associated with progressive infiltration of macrophages into adipose tissue. These adipose tissue macrophages (ATM) are referred to as classically-activated M1 macrophages and are potent sources of the cytokines IL-1 β , IL-6 and TNF α , which augment adipose tissue inflammation. While HFD undoubtedly drives macrophage recruitment, age is another factor that may play a role in this phenomenon. In this study we hypothesized that macrophage recruitment into adipose may be evident with age but the inflammatory

phenotype of ATM would be attenuated due to lack of pro-inflammatory high-fat insult.

Materials and methods: C57BL/6 mice (6–8wks old) were fed HFD (45% palm oil) or were maintained on a chow diet (CD) (10% palm oil) for 16 weeks. Glucose (GTT, 1.5g/kg glucose) and insulin (ITT, 0.75U/kg) tolerance tests were performed at baseline (6–8wks) and after dietary interventions (6mths old). Adipose was harvested from mice at baseline and after chow or HFD and cytokine secretion from adipose explants monitored after 24h in culture. Epididymal adipose tissue (EAT) was harvested at baseline or after 16wks HFD/CD. Presence of M1 macrophages (F4/80⁺CD11b⁺CD11c⁺) in the stromal vascular cells (SVCs) of adipose was analyzed by flow cytometry. Separately, a population of F4/80⁺CD11b⁺ cells were isolated from the SVCs of HFD/CD fed mice after 16wks dietary intervention and were cultured for 24hours *ex vivo* prior to RNA isolation. mRNA expression of inflammatory genes was assessed by real-time PCR. SVCs isolated from chow-fed or HFD-fed derived adipose tissue were co-cultured with 3T3L1 adipocytes for 48h. Insulin stimulated glucose transport into adipocytes and mRNA expression of key inflammatory and insulin signalling genes were monitored by real-time PCR.

Results: M1 macrophage recruitment increased equivalently from baseline ($4.53 \pm 1.7\%$) after HFD ($18.75 \pm 1.3\%$) and CD ($15.63 \pm 1.2\%$). Development of IR was only evident in HFD-fed animals with preservation of glucose tolerance in CD mice. Cytokine secretion from adipose tissue explants was markedly higher after HFD compared to CD despite presence of similar numbers of ATM. F4/80⁺CD11b⁺ cells isolated from mice fed a HFD exhibited greater mRNA expression of pro-inflammatory M1 markers IL-6, TNF α , IL-1 β , IL-18 and Nos2 compared to CD-derived cells. Furthermore co-culture of SVCs derived from HFD, but not chow-fed mice, with 3T3L1 adipocytes resulted in significantly reduced insulin-stimulated glucose uptake into adipocytes ($p < 0.05$) and enhanced adipocyte IL-6 and reduced IRS-1 and adiponectin mRNA.

Conclusion: In the present study we demonstrate that both obesity and age are associated with increased infiltration of M1 macrophages into adipose tissue but development of IR was only evident in high fat-fed cohorts. Further characterization revealed that the immunogenic phenotype of recruited ATM and adipose tissue explants was significantly higher after HFD compared to CD. Furthermore, SVCs from high-fat fed animals induced a greater degree of IR in co-cultured adipocytes compared to chow-derived cells. These results suggest that it is not the number of macrophages that infiltrate adipose that dictates severity of IR but the immunogenic phenotype of the recruited cells

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PS 057 Adipokines

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Endocannabinoids regulate adipokine production and the immune balance of omental adipose tissue in human obesity

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Background and aims: The endocannabinoid system (ES) is involved in regulation of energy homeostasis and the metabolism of lipids and glucose through central and peripheral pathways. Blockade of the cannabinoid type 1 (CB₁) receptor with Rimonabant in obese patients promoted significant decrease of bodyweight and waist circumference, and improvement in cardiovascular risk factors. The ensuing rise of circulating adiponectin (ApN) levels was not merely explained by weight loss and suggested specific improvement of adipose tissue function and immune regulation. The aim of this work was to address whether modulation of CB1 directly regulates the production of ApN and other potential adipokines by omental adipose tissue (OAT) of obese subjects.

Materials and methods: OAT was obtained from 30 obese subjects (BMI, 40.6 ± 1.3 kg/m²) undergoing abdominal (mainly bariatric) surgery. Tissue explants (2–3 mm³) or isolated adipocytes or stromal-vascular cells (SVC) were cultured for 24 h with or without a selective CB1 antagonist or a selective agonist (arachidonyl-2-chloroethanolamide; ACEA). mRNA levels of adipokines, which were previously found to be differentially regulated between lean and obese subjects, were measured by RTQ-PCR, and ApN secretion in medium was quantified by RIA. Phosphorylation of p38 Mitogen-activated protein kinase (MAPK) was analysed by Western blot.

Results: In adipose tissue explants, the CB1 blocker rimonabant upregulated ApN gene expression by ~40%. mRNA abundance of omentin which, like ApN, exhibits insulin-sensitizing properties and is decreased in obesity was upregulated as well (~ +70%). Conversely, mRNA levels of 2 pro-inflammatory cytokines, macrophage inhibitory protein (MIP)-1 β and interleukin (IL)-7 were downregulated (~-26% and ~-32%, respectively). We next examined where these effects took place within OAT. CB1 expression was roughly similar in both cellular fractions. In isolated mature adipocytes, blockade of CB1 reproduced the increase of ApN mRNA and the decrease of IL-7 mRNA while inducing a moderate but significant increase of ApN secretion into the medium (+20%). In isolated SVC, gene expression of omentin, which is restricted to this fraction, was augmented while that of MIP-1 β was diminished. To decipher the mechanisms leading to ApN regulation by the ES, we investigated several signalling pathways in adipocytes. The upregulation of ApN by CB1 blockade was unaltered by inhibiting cAMP production. Unlike the blockade, activation of CB1 by ACEA did down-regulate ApN gene expression, an effect reversed by specific inhibitors of p38MAPK (but not of JNK or ERK1/2). Western blot analysis further confirmed that ACEA did actually increase p38MAPK phosphorylation in adipocytes.

Conclusion: The ES directly regulates the immune balance of OAT in human obesity. Specifically, blockade of CB1 attenuates the inflammatory state in both cellular fractions of OAT either by increasing ApN and omentin production or decreasing mRNAs of MIP-1 β and IL-7. ApN regulation by the ES partly involves p38MAPK.

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N-3 polyunsaturated fatty acids lead to an increased production of lipid derived adipokines and improve systemic inflammation in severely obese subjects

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Background and aims: The systemic inflammatory response to obesity originates in the adipose tissue (AT) and is tightly linked to insulin resistance and type 2 diabetes. Eicosanoids are potent lipid mediators that play a key role in the regulation of inflammation. Such lipid mediators, called lipid-derived adipokines (LDA), are produced by the AT in considerable amounts. Whereas pro-inflammatory eicosanoids deriving mainly of the n-6 polyunsaturated

fatty acid (PUFA) arachidonic acid (AA) have been extensively studied, some highly potent n-3 PUFA-derived LDA and LDA-precursors have been identified only recently. Such novel LDA like 17-hydroxy-DHA (17-HDHA), resolvins and protectins have been showed to promote the resolution of inflammation. Since chronic inflammation could result from a failure to resolve an inflammatory response, we aimed to identify LDA production in AT from severely obese subjects and to investigate whether n-3 PUFA supplementation would impact LDA production, systemic inflammation, and insulin sensitivity.

Materials and methods: Fifty-four severely obese (BMI ≥ 40 kg/m²) non-diabetic patients, supposed to undergo elective bariatric surgery, were treated for two month with either 3,3 g/day highly purified n-3 PUFA (1,8 g EPA/1,5 g DHA) or an equivalent amount of mainly saturated fatty acids as a control, in a randomized, open label controlled clinical study. Compliance was assessed by comparing levels of EPA and DHA in plasma phospholipids before and after treatment by GC/MS. Plasma concentration of inflammation markers such as IL-6 and hsCRP were determined at baseline and at the end of treatment. Concomitantly, 2 h OGTT were performed and insulin sensitivity/resistance indices such as OGIS, CLIX, HOMA-IR were calculated. During the surgical procedure, omental AT samples were collected. LDA concentrations in AT were analysed by solid phase extraction and HPLC-MS/MS. For statistical analyses, we used repeated measures ANOVA, unpaired Student's t-test and Pearson's correlation.

Results: Plasma EPA and DHA levels increased after n-3 PUFA treatment (both $P < 0.001$). The intervention led to a decrease in AA concentration ($P = 0.03$) as well as a consistent improvement of the anti-inflammatory index AA/EPA ($P < 0.001$). Omental AT production of 17-HDHA and Protectin D1 was higher in the n-3 PUFA group ($P = 0.02$ and $P = 0.002$ respectively). Resolvin E1 and Resolvin D1 were only detectable in AT of n-3 PUFA treated patients. In this group we also found a correlation between AT 17-HDHA concentration and CLIX ($r = 0.82$, $P < 0.001$). The treatment lead to a decrease in IL-6 concentration ($P = 0.03$), without affecting hsCRP levels. No treatment-related changes were observed in any of the calculated insulin sensitivity/resistance indices.

Conclusion: Our results show that n-3 PUFA treatment of severely obese patients leads to a shift in LDA towards an increase in the synthesis of anti-inflammatory or resolving lipid mediators and their precursors in AT. These local changes were associated with beneficial effects on systemic inflammation. Although we could not detect any improvement in insulin sensitivity under the given conditions, the anti-inflammatory changes induced by n-3 PUFA may be beneficial in long term treatment.

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Decreased lactoferrin expression in adipose tissue and adipocytes in association with obesity

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Background and aims: Recently, a possible role of lactoferrin in adipogenesis and insulin action has been reported. To gain insight in this role, we aimed to investigate lactoferrin and delta-lactoferrin gene and protein expression in human adipose tissue and adipocytes in association with obesity.

Materials and methods: Lactoferrin and delta-lactoferrin gene expression was measured in 75 visceral and subcutaneous adipose tissue samples, in 16 adipose tissue fractions (Stromal vascular cells (SVCs) and adipocytes) and during adipocyte differentiation in human primary preadipocytes from lean and obese subjects. Lactoferrin protein levels were measured by elisa in adipose tissue and in adipocyte-conditioned media.

Results: Lactoferrin (LTF) gene and protein was expressed in both visceral and subcutaneous adipose tissue at similar levels as other master adipogenic genes such as PPAR γ . LTF gene expression was negatively associated with BMI and fat mass in both subcutaneous and visceral fat depots. In addition, LTF gene expression in subcutaneous adipose tissue was negatively associated with fasting glucose and fasting triglycerides and positively associated with lipogenic genes [FASN ($r = 0.67$, $p < 0.0001$), ACC1 ($r = 0.42$, $p < 0.0001$) and SCD1 ($r = 0.42$, $p < 0.0001$)]. LTF gene expression was significantly increased in adipocytes in comparison with stromal vascular cells (SVCs) (0.034 ± 0.01 vs. 0.001 ± 0.0003 R.U., $p = 0.03$). According with this, lactoferrin

levels increased during human adipocyte differentiation in parallel to adipogenic genes, and its levels were increased in human adipocytes from lean in comparison with obese subjects (0.008 ± 0.002 vs. 0.0007 ± 0.0001 , $p < 0.001$). The inhibition or stimulation of adipogenesis during adipocyte differentiation (with metformin or rosiglitazone administrations) reduced or increased, respectively, lactoferrin levels during adipocyte differentiation. Otherwise, delta-lactoferrin (δ -LTF) was expressed at low levels in human adipose tissue and adipocytes. δ -LTF gene expression was not associated with metabolic parameters, and it was more expressed in SVCs in comparison with adipocytes (0.018 ± 0.004 vs. 0.007 ± 0.002 R.U., $p = 0.03$). In human adipocytes, LTF but not δ -LTF gene expression increased in response to inflammatory conditions (TNF- α and LPS-macrophage conditioned medium) and rosiglitazone treatment. Lactoferrin protein levels in adipose tissue and circulating lactoferrin concentration in adipocyte conditioned medium run in parallel with LTF gene expression.

Conclusion: Lactoferrin gene and protein expression in adipose tissue was inversely associated with obesity, and increase during adipogenesis. These results suggest a beneficial role of lactoferrin improving insulin sensitivity and obesity-associated metabolic disturbances.

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Serum concentrations and adipose tissue expression of pigment epithelium-derived factor in obese patients with type 2 diabetes mellitus: the influence of very-low-calorie diet

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Background and aims: Pigment epithelium-derived factor (PEDF) is a novel adipokine and metabolic regulator with diverse effects on glucose homeostasis, inflammation and angiogenesis. The aim of the present study was to explore the possible role of PEDF in the positive metabolic effects of short term very-low-calorie diet (VLCD) in obese patients with type 2 diabetes mellitus (T2DM).

Materials and methods: Thirteen obese females with T2DM and 17 healthy lean sex- and age-matched controls (C) were included into the study. Serum concentrations of selected biochemical, hormonal and immunological parameters were measured by standard laboratory methods. The expression analysis of genes for PEDF, TNF- α , IL-6, IL-8, adiponectin and adiponectin receptor 1 and 2 in subcutaneous adipose tissue (SCAT) was performed by real-time PCR at baseline and after 3 weeks of VLCD (energy intake 2500 kJ/day).

Results: Compared to C group T2DM patients had significantly increased BMI (57.7 ± 2.7 vs. 22.2 ± 0.5 kg/m², $p < 0.001$), fasting glucose (8.51 ± 1.11 vs. 4.58 ± 0.11 mmol/l, $p < 0.001$), insulin (35.3 ± 3.7 vs. 17.3 ± 1.4 mIU/l, $p < 0.001$), CRP (2.25 ± 0.43 vs. 0.32 ± 0.12 mg/l, $p = 0.001$), TNF- α (6.53 ± 0.84 vs. 3.52 ± 0.39 pg/ml, $p = 0.017$), IL-6 (2.34 ± 0.45 vs. 1.01 ± 0.14 pg/ml, $p < 0.001$) and IL-8 (2.53 ± 0.26 vs. 1.67 ± 0.24 pg/ml, $p = 0.018$) and decreased concentrations of adiponectin (16.1 ± 2.0 vs. 26.4 ± 3.2 μ g/ml, $p = 0.012$). Serum PEDF was significantly elevated in T2DM group (16.7 ± 1.6 vs. 10.4 ± 0.5 ng/ml, $p < 0.001$) and correlated positively with BMI ($r = 0.633$, $p < 0.002$), fasting glucose ($r = 0.487$, $p = 0.025$), insulin ($r = 0.698$, $p < 0.001$), HOMA index ($r = 0.750$, $p < 0.001$), CRP ($r = 0.609$, $p = 0.004$) and IL-6 ($r = 0.592$, $p = 0.005$) in a combined population of T2DM and C patients. mRNA expression of PEDF, adiponectin and adiponectin receptor 2 was significantly ($p < 0.05$) reduced in SCAT of T2DM subjects, while no expression change was seen in other investigated parameters. Three weeks of VLCD markedly decreased body weight (57.7 ± 2.7 vs. 48.7 ± 2.3 kg/m², $p < 0.001$) and improved glycemia (8.51 ± 1.11 vs. 6.28 ± 0.64 mmol/l, $p = 0.008$), insulin resistance (HOMA index: 12.8 ± 1.5 vs. 8.1 ± 1.1 , $p < 0.001$) and inflammatory profile (CRP: 2.25 ± 0.43 vs. 1.31 ± 0.29 mg/l, $p = 0.01$; IL-6: 2.34 ± 0.45 vs. 1.53 ± 0.32 pg/ml, $p = 0.009$). PEDF serum concentrations (16.7 ± 1.6 vs. 14.7 ± 1.1 ng/ml, $p = 0.233$) and mRNA expression ($p = 0.113$) showed no significant change after VLCD. The same was true for mRNA expression of other studied factors.

Conclusion: Our results show that serum PEDF is increased in patients with T2DM and obesity and correlates well with nutritional status, parameters of glucose metabolism and inflammatory markers. The lack of change in serum concentrations or mRNA expression after VLCD does not support a significant role of PEDF in the positive metabolic effects of short-term caloric restriction.

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Retinol binding protein 4 and osteoprotegerin in patients with type 2 diabetes mellitus: relation to insulin resistance

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Background and aims: Adipose tissue has been demonstrated to secrete various adipokines that relate to insulin resistance, which has a causal role in type 2 diabetes mellitus (T2DM) and its cardiovascular complications. But understanding of the diverse effects of distinct adipokines and the interactions between these bioactive mediators and osteoprotegerin (OPG) as important regulatory molecule in atherosclerosis are still incomplete. Aim of this study was to evaluate OPG, retinol binding protein 4 (RBP4), other adipokines feature in T2DM patients exploring potential links between the above and insulin resistance parameters.

Materials and methods: A total of 60 patients with T2DM (M/F: 34/26, age 53.93±1.20 yrs, diabetes duration 6.29±0.67 yrs, BMI 32.68±0.77 kg/m², HbA_{1c} 7.06±0.18%) and 21 sex- and age matched healthy control subjects (C) were enrolled in the study. The plasma fasting OPG, adipokines (RBP4, total and HMW adiponectin, vaspin, fetuin-A, resistin, progranulin) and insulin levels were measured with ELISAs. The homeostasis model assessment of insulin resistance (HOMA-IR) and insulin sensitivity (QUICKI) were calculated. Fasting blood glucose, HbA_{1c}, free fatty acids (FFA), lipid profile, high sensitivity CRP(hs-CRP) and IL-6 were determined. The relationship between plasma OPG, RBP4, other adipokines and insulinemic state and metabolic variables was also analyzed (Spearman correlation). Data were presented as mean ± SEM.

Results: Comparing with C significant increase in triglyceridemia (3.29±0.41 vs. 1.56±0.20 mM/L), plasma FFA levels (1.08±0.07 vs. 0.70±0.06 mM/L), HOMA-IR (8.01±0.76 vs. 4.53±0.58) as well as strongly marked decrease in adiponectinemia (total adiponectin 6.22±0.33 vs. 11.80±1.45 mg/L; HMW adiponectin 2.70±0.20 vs. 6.80±0.91 mg/L) and impaired insulin sensitivity (QUICKI 0.47±0.01 vs. 0.50±0.01) were observed in T2DM patients. Plasma RBP4 and OPG levels were significantly ($p<0.001$) higher in T2DM patients in comparison with C (33.28±0.99 vs. 23.01±1.82 mg/L; 493.00±38.80 vs. 312.56±24.78 pg/ml, respectively), hs-CRP and IL-6 were also increased. No differences were observed in resistin, vaspin, fetuin-A and progranulin. In T2DM patients, a strong linear relationship was revealed between OPG value and both progranulin ($r=0.419$, $p<0.002$) and fetuin-A ($r=0.367$, $p<0.001$); there was a correlation with RBP4 ($r=0.253$, $p<0.032$) and resistin ($r=0.301$, $p<0.01$), but not with fasting glucose, HbA_{1c}, insulin or adiponectin (total and HMW). It was identified a relationship of RBP4 to fasting insulin ($r=0.440$, $p<0.004$) and HOMA-IR ($r=0.501$, $p<0.001$) as well as inverse correlation with insulin sensitivity ($r=-0.501$, $p<0.001$). A significant positive correlation was found between RBP4 and progranulin.

Conclusion: In T2DM patients, circulating novel adipokine RBP4 was increased and correlated positively with insulin resistance. Furthermore, it was found significant enhancement of OPG and direct relationship between RBP4 and OPG in circulation. These findings provide a rationale for antidiabetic therapies aimed at lowering plasma RBP4 and OPG levels.

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Relationship between retinol binding protein 4 and the risk of cardiovascular diseases

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Background and aims: Visceral obesity has been suggested to be an independent risk factor for cardiovascular disease (CVD); the role of adipokines in the risk for CVD is less clear. Aim of this study was to investigate the relationship between parameters of visceral obesity and index of CVD risk.

Materials and methods: A cross-sectional analysis of healthy males ($n = 116$) and females ($n = 175$) for evaluation of clinical, laboratory, and anthropo-

metric parameters were undertaken. Abdominal subcutaneous and visceral adipose tissues (SAT and VAT) were measured by computed tomography. Adipokines, including RBP 4 and adiponectin, were determined. The risk for CVD was estimated using the 10-year Framingham Coronary Heart Disease Risk Point scale (10-y FCRP)

Results: The 10-y FCRP was significantly correlated with VAT ($\gamma = 0.123$, $P = 0.049$) and RBP4 ($\gamma = 0.230$, $P < 0.001$), after adjustment for age, gender and BMI. The magnitude of the increased the 10-y FCRP correlated with VAT and RBP4 independent of obesity. In a multiple linear regression model, the serum levels of RBP4 at a given body mass index (BMI) and VAT significantly correlated with the 10-y FCRP ($\beta = 0.123$, $P < 0.001$).

Conclusion: RBP4 is closely associated with the 10-y FCRP. RBP4 appears to provide a link between central obesity and CVD.

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Effect of short-term hyperglycaemia on the release of adipocytokines from subcutaneous adipose tissue in obese women

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Background and aims: Diabetes mellitus type 2 is characterized by a pro-inflammatory state associated with an increase of circulating levels of adipocytokines and chemokines. The aim of this study was to investigate whether hyperglycemia per se may increase the release of adipocytokines/chemokines from adipose tissue and, thus, contribute to their increased levels in circulation.

Materials and methods: 9 healthy obese women (age 35+/-5, 1yr, BMI 34+/-4, 9kg/m²) underwent 3 hours' hyperglycemic clamp (HGC) with infusion of somatostatin (to inhibit the endogenous insulin release). The net output of interleukin 6 (IL6) and monocyte chemoattractant protein-1 (MCP-1) from subcutaneous abdominal adipose tissue (SCAAT) was assessed by measurements of arterio-venous differences at rest and during HGC. Blood samples were taken from vein draining subcutaneous abdominal fat and compared with arterial blood samples. Adipose tissue blood flow (ATBF) was assessed using the local Xe-clearance technique.

Results: There was no change of ATBF during HGC (baseline: 1,56+/-1,34 vs 3rd hour of HGC: 1,39+/-0,58 ml/100 g/min, NS). The net outputs of IL-6 and MCP-1 from SCAAT were higher at the end of HGC when compared with baseline (IL 6: 4,2+/-5,28 vs 18,08 +/- 16,44 ml/100 g/min, $p<0,05$; MCP-1: 13,68+/- 31,99 vs. 63,33 +/- 50,73 ml/100 g/min, $p<0,01$). In arterial blood there was a non significant tendency to an increase during HGC for IL-6 (1,70+/-0,89 vs. 3,42+/-3,26 pg/ml, NS) and for MCP-1 (190,28+/- 83,75 vs 211,95+/- 86,83 pg/ml, NS).

Conclusion: Short-term hyperglycemia induces an increase in the release of IL-6 and MCP-1 from SCAAT and, thus, may play a role in the development of the low-grade inflammatory state in adipose tissue associated with diabetes mellitus type 2.

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The novel adipokine DPP4 exhibits a stronger release from visceral human adipose tissue compared to subcutaneous adipose tissue in association with insulin resistance

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Background and aims: By an unbiased proteomic approach, we could recently identify dipeptidyl peptidase 4 (DPP4) as a novel adipokine. DPP4 cleaves N-terminal dipeptides from various substrates including incretins. DPP4 inhibitors prolong the insulinotrophic effect of GLP1 and are in clinical use. DPP4 expression and release increases during adipogenesis of human adipocytes. *In vitro*, DPP4 induces insulin resistance in adipocytes and muscle cells. Human subcutaneous adipose tissue (AT) explants release DPP4 correlating positively with donor BMI, fat cell volume and the metabolic syn-

drome. Circulating DPP4 is significantly increased in obese patients as compared to lean controls and positively correlated with fat cell size and leptin. This study is aimed to 1) assess depot-specific differences of DPP4 release in lean, obese and diabetic patients, 2) measure *in vivo* release of DPP4 from subcutaneous abdominal AT and 3) compare circulating DPP4 levels in insulin-sensitive obese and matched insulin-resistant obese.

Materials and methods: Paired AT explants from the subcutaneous and visceral depot were generated from lean controls and lean diabetic patients as well as obese controls and obese diabetic subjects and DPP4 release was measured by ELISA. Arterio-venous differences in circulating DPP4 were measured across subcutaneous abdominal AT in 17 lean and obese volunteers. 60 morbidly obese patients divided into an insulin-sensitive and insulin-resistant group based on euglycemic hyperinsulinemic clamp and matched for age, gender and body fat, were analyzed for serum DPP4.

Results: DPP4 release was significantly increased from visceral AT explants compared to subcutaneous AT in lean and obese subjects (5-fold and 7-fold, respectively). The highest DPP4 release could be measured from visceral AT of obese diabetic patients compared to lean and non-diabetic controls. A net release of DPP4 from AT also occurred under *in vivo* conditions with obese subjects and women exhibiting an increased net release from subcutaneous abdominal AT. Insulin-sensitive obese patients are characterized by significantly lower circulating DPP4 as compared to obesity-matched insulin-resistant patients. In this cohort, DPP4 positively correlates with the amount of visceral AT, HbA1c, % macrophages in omental AT and adipocyte size. DPP4 is not correlated to total body fat and the amount of subcutaneous AT.

Conclusion: DPP4 is a novel adipokine with higher release from visceral adipose tissue that is particularly pronounced in obese diabetic patients. In obese patients, circulating DPP4 is associated with insulin resistance and correlated with visceral AT mass, enlarged adipocytes and adipose tissue inflammation. Our data suggest that DPP4 might be a biomarker for visceral obesity and the metabolic syndrome.

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Serum visfatin is differentially regulated by insulin and non-esterified fatty acids in healthy men

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Background and aims: Visfatin is a protein secreted by adipose tissue which was discovered as a protein with insulin-mimetic properties. Experimental and clinical studies demonstrated that visfatin can be involved in the pathogenesis of insulin resistance. It was demonstrated that plasma visfatin is elevated in insulin resistant states i.e. obesity, type 2 diabetes mellitus and polycystic ovary syndrome. In vitro study showed that insulin inhibits visfatin release from adipocytes. The aim of the present study was to evaluate serum visfatin concentration during hyperinsulinemia (6-hours hyperinsulinemic euglycemic clamp) and then during insulin resistant conditions caused by an acute elevation of NEFA (6-hours hyperinsulinemic clamp combined with Intralipid-heparin infusion).

Materials and methods: The study group consisted of 19 apparently healthy male volunteers (mean age 25 ± 7 years, BMI 24.3 ± 3 kg/m²). Clinical examination, anthropometric measurements, OGTT, plasma lipids and liver enzymes activity were measured. Subjects underwent 6h euglycemic hyperinsulinemic clamp and after one week 6h hyperinsulinemic euglycemic clamp combined with Intralipid-heparin infusion. Measurements of serum visfatin during both clamp studies were performed.

Results: 6-hours of insulin infusion during clamp resulted in significant decrease in serum visfatin concentration ($p=0.0057$), however after 2h there was no change in serum visfatin concentration. Concomitant 6-hours Intralipid-heparin infusion which caused a significant increase in NEFA concentration ($p<0.0001$), resulted in marked increase in serum visfatin ($p=0.00035$) which was already observed after 2 hours of Intralipid infusion ($p=0.00028$). Serum visfatin during clamp study after 2h and 6h of Intralipid infusion were significantly higher than the respective values during clamp study without elevation of NEFA (both $p<0.0001$). The increase of serum visfatin during Intralipid infusion (delta visfatin) was positively related to body weight ($r=0.54$, $p=0.016$), %body fat ($r=0.48$, $p=0.036$) and gamma glutamyl transpeptidase ($r=0.56$, $p=0.011$).

Conclusion: Our data show that plasma visfatin is differentially regulated by insulin and NEFA. One might suggest that induction of insulin resistance by NEFA suppress insulin inhibition of visfatin production by adipose tissue, resulting in plasma visfatin increase in insulin resistant conditions.

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Molecular mechanism of the insulin sensitizing adipokine vaspin

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Background and aims: Obesity significantly increases the risk of developing type 2 diabetes mellitus, hypertension, coronary heart disease, stroke, and several types of cancer. Vaspin (visceral adipose tissue-derived serpin) was identified as an adipokine with insulin-sensitizing effects, which is predominantly secreted from visceral adipose tissue in a rat model of type 2 diabetes. Recently, we reported that elevated vaspin serum concentrations are associated with obesity and impaired insulin sensitivity in humans. Therefore, it has been postulated that increased vaspin expression and secretion could represent a compensatory mechanism associated with obesity, severe insulin resistance, and type 2 diabetes. Although, antiprotease properties have been suggested as mechanism of vaspin action, until now a protease substrate of vaspin has not been identified.

Materials and methods: We recombinantly expressed human vaspin for crystallization and *in vitro* protease inhibition screenings. C57BL/6J and db/db mice were treated with recombinant vaspin to investigate vaspin effects on glucose tolerance and insulin sensitivity. Expression of vaspin and the target protease were investigated in tissue samples using immunohistochemistry and in human serum samples.

Results: Here, we show that the crystal structure of vaspin confirms the typical serpin structure and suggests a protease target. We find that vaspin is expressed in pancreatic beta cells and inhibits a member of the kallikrein family with a high specificity via typical serpin mechanism *in vitro*. This is the first identified target of vaspin. Consistent with a potential insulin protective role of vaspin, plasma insulin concentrations in response to glucose are higher in mouse models treated with recombinant vaspin compared to controls explaining the glucose lowering effect of vaspin. Patients with type 2 diabetes show significantly lower kallikrein serum concentrations. Together with increased vaspin expression in obesity and type 2 diabetes these data corroborate the vaspin-kallikrein system as a physiological compensation mechanism in the metabolically challenged state of obesity and insulin resistance.

Conclusion: Our results suggest the vaspin-kallikrein system as a potential novel target for anti-diabetic treatment strategies.

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PS 058 Adiponectin

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Adiponectinaemia, metabolic status, insulin secretion and insulin resistance in obese women: influence of weight loss

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Background and aims: Obesity is strongly associated with type 2 diabetes mellitus and prediabetes. Loss of the excessive fat mass is expected to improve glucose metabolism. Adiponectin is an adipokine which levels increase with fat mass loss. AIMS: 1) To compare variation (Δ) in adiponectin levels, in insulin secretion (IS) and insulin resistance (IR) indexes, and in anthropometric parameters and metabolic status changes in obese women during weight loss program, according to their initial status; 2) to assess the association between changes in adiponectin levels, IS and IR indexes with those in anthropometric parameters.

Materials and methods: We studied 100 premenopausal obese women anthropometrically characterized. They were submitted to fasting blood sample collection, followed by an oral glucose tolerance test (oGTT). We used one index of IR (homeostatic model -HOMA-IR), one of insulin sensitivity (Matsuda formula) and two IS indexes (homeostatic model -HOMA%beta and insulinogenic index -INS-i). Women entered in a weight loss program and were reassessed for all parameters after 6 and 12 months.

Results: Patients were characterized by mean age=34.1±8.2 yrs, BMI=43.6±8 Kg/m², fat mass=54±15.1 Kg, waist circumference=117.7±15.2cm, waist:hip ratio (WHR)=0.88±0.08, adiponectin=6.86±3.21µg/ml, Matsuda=3.69±2.54, HOMA-IR=4.42±3.65, HOMA%beta=262.1±174.9 and INS-i=24.2±20.2. Normoglycemia (NG) was present in 71 patients, prediabetes in 19 and diabetes in 10. Eighty six patients were reassessed at month 6 and 67 accomplished the 12 months follow-up. No difference between metabolic status groups were observed for anthropometric or adiponectin variation between baseline and months 6 or 12. There was a significantly difference in metabolic status characterization between baseline and month 6 ($p<0.001$) and between baseline and month 12 ($p<0.001$). Between baseline and month 6 we observed that Δ HOMA-IR was positively correlated with Δ waist ($p<0.001$; $r=0.475$) and Δ fat mass ($p<0.001$; $r=0.579$); Δ Matsuda were negatively correlated with Δ waist ($p<0.001$; $r=-0.473$), Δ WHR ($p=0.006$; $r=-0.333$) and Δ fat mass ($p<0.001$; $r=-0.521$); Δ INSi were negatively correlated with Δ waist ($p<0.001$; $r=-0.427$), Δ WHR ($p=0.001$; $r=-0.385$) and Δ fat mass ($p=0.002$; $r=-0.379$); we found a direct correlation between Δ adiponectin and Δ Matsuda ($p=0.003$; $r=0.361$). Considering variations between baseline and month 12, we observed that Δ HOMA-IR was positively correlated with Δ waist ($p<0.001$; $r=0.792$), Δ WHR ($p<0.001$; $r=0.547$) and Δ fat mass ($p<0.001$; $r=0.76$); Δ Matsuda were negatively correlated with Δ waist ($p=0.002$; $r=-0.479$), Δ WHR ($p=0.015$; $r=-0.393$) and Δ fat mass ($p=0.005$; $r=-0.442$). Δ adiponectin was negatively associated with Δ waist ($p<0.001$; $r=-0.448$) and Δ fat mass ($p<0.001$; $r=-0.568$); however it was independently and inversely correlated with Δ HOMA-IR ($p=0.020$; $r=-0.367$).

Conclusion: In weight loss programs, obese women lose fat mass (with a subsequently increase in insulin sensitivity and insulin secretion) independently from their initial metabolic status. Adiponectin levels parallels insulin sensitivity indexes during the process of weight reduction, independently from the amount of fat mass loss. That might indicate a direct insulin-sensitizing effect of adiponectin.

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Adiponectin improves endothelial dysfunction caused by elevated FFAs levels, partly through cAMP-dependent pathway

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Background and aims: Elevation in circulating free fatty acids (FFAs) is a characteristic metabolic abnormality of the insulin-resistant state, which may be causally related to the onset and progression of endothelial dysfunction. Adiponectin may improve endothelial function in obese rats by reducing oxidative/nitrative stress and regulation of eNOS activity. However, whether adiponectin may protect endothelial cells and attenuate endothelial dysfunction caused by elevated FFAs concentration remains unknown. The current study aimed to determine whether adiponectin may independently improve

the endothelial dysfunction caused by high FFAs concentration and the role of cAMP in the action of adiponectin on endothelial cell.

Materials and methods: Male Sprague-Dawley rats (10 weeks old) thoracic aortas were isolated then cut into four rings of 3-mm length, and was incubated in organ bath contained 20ml of Krebs-Henseleit buffer with different agents separately: 800µmol/L FFA (F, n=14); 800µmol/L FFA+ 2µg/ml adiponectin (A, n=14); 800µmol/L FFA+ 2µg/ml adiponectin + 200µmol/L adenylate cyclase inhibitor dideoxyadenosine (D, n=7); blank control (N, n=10). After 2h of incubation, the aortic rings were precontracted with norepinephrine (0.1µmol/L), then the rings were exposed to cumulative concentration of Acetylcholin (Ach, 10^{-9} - 10^{-5} mol/L) or sodium nitroprusside (SNP, 10^{-10} - 10^{-6} mol/L). Endothelial dysfunction was defined as a reduced vasorelaxation in response to Ach with a normal response to SNP. The NF-κB expression was evaluated immunohistochemically in rat aortic section.

Results: Acetylcholine caused a concentration dependent vascular relaxation in all pre-constricted aortic rings. At the maximal concentration, Ach induced (96.0±2.9)% of vasorelaxation in the N group, indicating well preservation of endothelial function during our procedure. The vasorelaxation was significantly reduced in the F group in comparison with the N group [(60.9±6.7)vs (96.0±2.9)% ($p<0.05$)]. Pretreatment of adiponectin dramatically although incompletely restored maximal vascular relaxation response to Ach [(88.5±3.3) vs (96.0±2.9)% ($p<0.05$)]. Furthermore this vascular protective effect was partly abolished by cAMP signal pathway inhibitor dideoxyadenosine, maximal endothelial dependent vasodilation of the D group reduced to (78.4±6.8)% ($p<0.05$). SNP induced vasodilation had no statistical significance among four groups. Increased NF-κB expression was noted in F group when compared with N group. Pretreatment with adiponectin partly decreased NF-κB expression when compared with F group.

Conclusion: This study demonstrated that adiponectin may independently mitigate endothelial impairment caused by high FFA concentration, and this effect was partly abolished by cAMP inhibitor. These finding raised the possibility that adiponectin perform its vascular protective function partly through activating cAMP signaling pathway, subsequently inhibiting NF-κB expression, which is known to play a central role in the regulation of inflammatory reactions in various types of cells.

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Maternal adiponectin multimers determine cord blood adiponectin concentrations

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Background and aims: Adiponectin (Adp) is an adipokine that has been proposed to play a role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues. It is secreted into bloodstream in three different multimeric forms (HMW, MMW, LMW) and the amount and distribution of these forms seem to determine its activity. It is present during fetal life and its levels increase markedly until delivery. Little is known about the factors that determine the concentration of the different multimeric forms during fetal life. The aim of this study was to analyze the influence of Gestational Diabetes Mellitus (GDM) on cord blood adiponectin (cb-Adp) concentrations in full term neonates, and their relationship with some clinical and analytical maternal and neonatal parameters.

Material and methods: 212 women with a singleton pregnancy, 132 with normal glucose tolerance (NGT) and 80 with GDM, and their offspring were studied. Women were recruited between 26 and 30 week of pregnancy at the time of the oral glucose tolerance test and were followed-up until delivery. Cord blood (cb) was obtained at delivery. Maternal and neonatal clinical and anthropometrical data were recorded. Total Adp and the multimeric forms of Adp were determined in cb and maternal (m) serum by a human ELISA kit (Multimeric Adiponectin ELISA Kit; Bühlmann, Schönenbuch, Switzerland). Fasting insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR). Spearman correlation coefficient was performed to assess the relationship between cb-Adp levels and clinical and analytical parameters. A stepwise linear regression analysis was used to identify the variables independently related to cb-Adp levels.

Results: maternal age, pre-pregnancy BMI, gestational age at delivery, birth weight (BW) and ponderal index (PI) were similar in the NGT and GDM groups. Total and HMW-Adp levels in maternal serum, were lower in the

GDM compared to the NGT group; Insulin concentrations and HOMA-IR were higher in GDM patients. No differences in Adp levels or its multimeric forms were observed in cb of offspring of diabetic mothers compared to NGT ones, but cb-insulin levels were higher in the GDM group. Cb-Total Adp and cb-HMW Adp levels were positively correlated with BW and PI, whereas cb-MMW Adp was negatively correlated with PI. Interestingly, cb-Adp levels and the multimeric forms of Adp were positively correlated with m-Adp concentrations in bivariate analysis and this relationship persisted after adjustment for GDM, age, pre-pregnancy BMI, gain in BMI and HOMA-IR. In the multivariate analysis, m-MMW Adp (B:3.397; $p<0.001$), PI (B:4920.88; $p<0.01$) and m-LMW Adp (B:1.194; $p<0.05$) were independent predictors of cb-Total Adp. m-MMW Adp (B: 2,399; $p<0.001$) and PI (B:3615,10; $p<0.05$) were predictors of cb-HMW Adp; m-MMW Adp (B:1.038; $p<0.001$) and m-HMW Adp (B:-0.225; $p<0.01$) were the best independent predictors of cb-MMW Adp whereas cb-LMW Adp was only independently related to m-LMW Adp (B:1.075; $p<0.001$).

Conclusion: Cb-Adp levels are independently related to m-Adp concentrations and unrelated to the diagnosis of GDM. Maternal multimeric forms of Adp at the beginning of the third trimester are the main independent predictors of the concentrations of cb-Adp and its multimeric forms at delivery.

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Cord blood adiponectin is an independent predictor of neonatal adiposity

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Background and aims: Adiponectin (Adp) is an adipose tissue derived protein that has been proposed to have a role in the modulation of foetal growth. Adp is detectable in cord serum as early as 24 weeks of pregnancy and its levels increase markedly until delivery coinciding with the period of greater adipose tissue accretion. Adp circulates in three different molecular forms, high molecular weight (HMW), medium molecular weight (MMW) and low molecular weight Adp(LMW). The amount and distribution of these forms seem to determine the activity in peripheral tissues. The aim of this study was to analyze the relationship of the different circulating forms of Adp with neonatal adiposity.

Materials and methods: 172 women with a singleton pregnancy, 102 women with normal glucose tolerance (NGT) and 70 women with Gestational diabetes mellitus (GDM) and their offspring were studied. Women were recruited between 26 and 30 week of pregnancy at the time of the 100 g oral glucose tolerance test and were followed-up until delivery. Cord blood (cb) was obtained from the umbilical vein at delivery. Maternal clinical and demographic characteristics, pregnancy outcome and neonatal anthropometric parameters were recorded. Total Adp and the multimeric forms of Adp were determined in cb by a human ELISA kit (Multimeric Adiponectin ELISA Kit; Bühlmann, Schönenbuch, Switzerland). Fasting insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR). Neonatal adiposity was assessed by the sum of skinfolds (SS) and the percent of body fat mass (PBFM) according to the formula proposed by Catalano. Spearman correlation coefficient was performed to assess the relationship between cb-Adp levels with neonatal parameters, and stepwise linear regression analysis were developed to identify if cb-Adp levels were independent predictors of birth weight (BW), ponderal index (PI), SS and PBFM. Each parameter was included as dependent variable and cb-Adp with other clinical and metabolic parameters as independent variables.

Results: Fasting glucose levels ($p<0.001$) and HOMA-IR ($p=0.010$) were higher in the GDM group compared to the NGT. Age, gestational age at entry in the study and pre-pregnancy BMI were similar in both groups. In terms of foetal outcome, there was no difference in gestational age at delivery, mean BW, PBFM, SS or PI. Umbilical insulin levels were significantly higher (4.33[2.35-6.87] vs 4.91[3.05-10.30]; $p=0.010$) in infants born from GDM women, whereas no differences were observed in total Adp levels and its multimeric forms. Cb-total and cb-HMW Adp were positively correlated with foetal parameters (BW, PI, SS and PBFM). Also cb-MMW Adp was positively correlated with SS and PI whereas LMW was positively correlated with all the parameters studied except for BW. In a stepwise multiple lineal regression analysis cb-Adp emerged as an independent predictor of PI (B:0.008; $p<0.01$), SS (B:0.068; $p<0.05$) and PBFM (B:0.102 ($p<0.01$)). Other variables include: pre-pregnancy BMI, BMI gain and cb Insulin. To identify which multimeric form was responsible of this association, the same models were developed

with the multimeric forms of Adp instead of Total Adp. cb-LMW and cb-MMW Adp emerged as independent predictors.

Conclusion: cb Adp levels are independent predictors of neonatal adiposity and the lower weight molecular multimeric forms of Adp seem to be the main responsible of this association.

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Gender differences in the response of adipose tissue to deleterious effects of high-fat feeding in mice

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Background and aims: Human as well as animal studies demonstrate less severe obesity-related metabolic disorders (metabolic syndrome) including peripheral tissue insulin resistance and dyslipidemia and/or later onset of these adverse conditions in female than in male subjects. However, mechanisms underlying a relatively low susceptibility of females to various components of the metabolic syndrome remain largely unknown. The goal of this study was to assess the role of adipose tissue in high-fat diet-induced impairment of glucose homeostasis in female as compared with male mice.

Materials and methods: Female and male mice of the C57BL/6N strain were fed from weaning either a chow or obesogenic high fat diet (HF; lipids ~35% wt/wt) for 15 or 35 weeks. Metabolic markers and hormones in plasma, glucose homeostasis, adipocyte size, inflammatory markers and cellularity of gonadal (gWAT) and subcutaneous (scWAT) adipose tissue depots were evaluated.

Results: Although HF-fed female mice were leaner as compared with males during the initial 20 weeks of the experiment, the body weight of both sexes equalized subsequently. Thus, at the end of experiment (Week 35), female mice exhibited greater weight gain and fat accumulation than males, a feature that was associated with larger adipocytes in both fat depots of female mice (~1.21-fold and ~1.19-fold increase in gWAT and scWAT, respectively). While adipose tissue macrophage infiltration, assessed as the number of crown-like structures (CLS) formed by aggregated macrophages, in response to HF feeding was in general less frequent in scWAT (Week 35), it was reduced in both fat depots of female as compared with male mice (~3.1-fold and ~2.8-fold reduction in the CLS number in gWAT and scWAT, respectively). Furthermore, later onset of impaired glucose homeostasis and better insulin sensitivity of female mice was associated with higher adiponectin levels in their circulation, largely independent of the type of diet and duration of the treatment (~1.5-fold difference between the sexes).

Conclusion: As compared to males, female mice display increased capacity for adipocyte enlargement in response to a long-term HF feeding, which is associated with a reduced infiltration of adipose tissue by macrophages and better insulin sensitivity in these mice. Our data suggest that adipose tissue expandability and adiponectin levels might play a role in the gender-specific differences in the development of obesity-associated metabolic disorders.

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Adiponectin inhibits nerve growth factor function through direct interaction

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Background and aims: Adiponectin, an adipokine, plays roles in regulation of metabolic effects and also prevents biological activity of some growth factors such as PDGF-BB through their direct interaction. Here, we examined possible interaction of adiponectin with nerve growth factor- β (NGF- β) being known as an adipokine.

Materials and methods: Direct interaction between adiponectin and NGF- β was examined by surface plasmon resonance method. The effect of adiponectin on NGF- β function was assessed by morphological changes in PC12 cells.

Results: Perfusion of increasing concentrations of NGF- β on the surface of full-length adiponectin-bound chip caused specific increase of mass bound, with the dissociation constant (KD) of 1.0×10^{-7} M and NGF- β /adiponectin ratio of 0.6. NGF- β also bound globular adiponectin-bound chip, but re-

duced KD value and the binding ratio. Treatment of PC12 cells with NGF- β , but not adiponectin, induced neurite outgrowth and cell swelling. However, treatment of the cells with NGF- β and adiponectin simultaneously decreased number of cells with neurite and cell size, compared with those of NGF alone. Treatment of the cells with adiponectin, but not NGF- β , increased activity-related site-specific phosphorylation of AMP-kinase α subunit (AMPK α), and the cells expressed Adipo R1 and Adipo R2 mRNAs. Treatment of the cells with siRNA for Adipo R1 and Adipo R2 prevented adiponectin-induced phosphorylation of AMPK α , but failed to affect adiponectin-caused inhibition of NGF- β -induced neurite outgrowth.

Conclusion: The results indicate that adiponectin interacts with NGF- β in solution, thereby inhibits NGF- β -induced neurite outgrowth and suggest that adiponectin modulates NGF- β function in the adjacent regions of adipocytes.

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The rab11 effector rab couplin protein (RCP) regulates adiponectin trafficking and secretion in adipocytes

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Background and aims: Adiponectin is an adipocytokine secreted by white adipocytes that regulates insulin sensitivity in peripheral tissues. Adiponectin synthesis and secretion is compromised in obesity and diabetes, resulting in decreased circulating serum levels. The intracellular machinery involved in mediating adiponectin release is largely unknown. We previously reported that intracellular adiponectin partially compartmentalises with rab5 and rab11, markers for the early/sorting and recycling compartments respectively. Here we aimed to investigate the role of several rab11 downstream effector proteins in regulating adiponectin trafficking and secretion.

Materials and methods: GFP-tagged rab11-FIP coding vectors were co-expressed with myc-tagged adiponectin into HEK293 cells or into 3T3L1 adipocytes and localisation of adiponectin-myc and FIP proteins was subsequently determined by immunofluorescent microscopy. Stable 3T3L1 cell lines carrying shRNA for RCP and RIP11 were generated by infecting 3T3L1 cells with lentiviral particles carrying FIP specific shRNA sequences or a scramble shRNA. Released and intracellular adiponectin content was determined using an ELISA kit.

Results: Overexpression of wild type rab11-FIP1 (RCP), FIP3 and FIP5 (Rip11) decreased the amount of secreted adiponectin expressed in HEK293 cells ($p<0.05$), whereas overexpression of rab11-FIP2 or FIP4 had no effect. In addition, the expression of RCP and Rip11 mutants unable to bind rab11 altered adiponectin secretion in HEK293 cells ($p<0.05$). This was supported by shRNA-mediated depletion of RCP and Rip11. Importantly, knock down of RCP in fully differentiated adipocytes also increased adiponectin secretion ($p<0.05$) whereas depletion of Rip11 had no effect in adipocytes.

Conclusion: In all, our findings identify RCP as a novel protein involved in the regulation of adiponectin trafficking and secretion through the endosomal compartments of fully differentiated adipocytes.

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Adiponectin levels negatively associate with beta cell function in type 1 diabetes, in contrast to leptin and resistin

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Background and aims: Adiponectin, leptin and resistin are fat tissue derived peptides with regulatory functions on metabolism and the immune system. A protective (adiponectin, leptin) or disease promoting (resistin) activity has been made probably for type 2 diabetes whereas the possible role of these adipokines in type 1 diabetes is unknown. We therefore

searched for an association with β -cell function in the context of a European multi-centre trial.

Material and methods: The European C-Peptide Trial included 118 patients (mean age 20.3 ± 7.5 years, 70 males) within less than 5 years from diabetes diagnosis. All participants underwent a standardised liquid mixed meal tolerance test (MMTT), and serum samples at -5 until 120 min were centrally analysed by multiplex technology for adipokines or standard procedures for metabolic parameters. Differences of C-peptide levels between patient groups were carried out using multiple regression test adjusted for sex, age, diabetes duration, BMI, baseline glucose, HbA1c or baseline C-peptide, where applicable.

Results: Serum concentrations of the three adipokines varied little over the 120 min of the MMTT. Patients were divided by their adipokine levels in subgroups above or below the median level ("higher versus lower"). Higher adiponectin levels ($>10.6 \mu\text{g/ml}$, mean $15.3 \mu\text{g/ml}$) were associated with lower fasting and stimulated C-peptide concentrations than seen in the lower adiponectin $<10.6 \mu\text{g/ml}$, mean $5.9 \mu\text{g/ml}$ subgroup (fasting C-peptide 0.10 vs 0.14 pmol/ml , $p<0.02$; stimulated C-peptide 0.18 vs 0.31 pmol/ml AUC , $p<0.03$). Conversely, higher leptin or resistin levels associated positively with fasting and stimulated C-peptide concentrations ($p<0.04$ for all comparisons). All differences remained significantly after adjustment for baseline metabolic parameters, including baseline C-peptide when analysing stimulated C-peptide (all $p<0.05$). Correlation analyses confirmed the negative association of adiponectin levels with fasting and stimulated C-peptide levels ($p<0.001$) versus positive associations for leptin and resistin (all $p<0.04$). There was no association between serum adiponectin levels and baseline insulin or HbA1c levels.

Conclusion: Serum adiponectin levels negatively associate with β -cell function in patients with type 1 diabetes. Contrary to what is known for type 2 diabetes, adiponectin does not seem to exert a protective effect on disease development in type 1 diabetes. Surprisingly, both leptin and resistin levels correlated with better preservation of β -cell function in early type 1 diabetes which may be mediated by modulation of innate immunity and regulatory T-cell functions by the two adipokines.

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Genetic variation in the genes encoding ADIPOQ and PPAR- γ influences on cardio-metabolic risk factors

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Aim: To investigate the effect of single nucleotide polymorphisms in the adiponectin and PPAR- γ genes on cardio-metabolic risk factors.

Materials and methods: Single nucleotide polymorphisms (SNPs) were genotyped for subsequent association studies on BMI, T2D and related metabolic traits in 752 men and women. Persons with no acute disease or severe chronic disorders underwent physical examination. Fasting blood sample was examined: lipids (total cholesterol, HDL-C, LDL-C, TAG), adiponectin (Adp), insulin; HOMA-IR was calculated. We used PCR to determine the 45T>G (rs2241766), 276C>T (rs 1501299) polymorphisms in adiponectin gene and PPAR- γ 2 Pro12Ala polymorphism. Regression analyses were used to find the associations of single nucleotide polymorphisms with cardio-metabolic risk factors.

Results: The frequency of SNPs PPAR- γ Pro12Ala, ADIPOQ +45T>G and +276G>T in the whole study population ($n=752$) and in the obesity subjects ($n=368$) is comparable (0,158 and 152; 0,064 and 0,058; 0,256 and 0,276, respectively). In women the PPAR- γ 2 Ala12 variant plays a protective role in hypercholesterolemia ($p=0,01$). T allele ADIPOQ rs2241766 is significantly associated with reduced risk of obesity ($p=0,004$). In men T allele ADIPOQ rs1501299 is consistently associated with obesity ($p=0,002$), abdominal obesity ($p=0,04$), prandial hyperglycemia ($p=0,002$); G allele ADIPOQ rs 1501299 plays a protective role for HDL cholesterol level ($p=0,047$).

Conclusion: The allelic variation of the genes encoding adiponectin and PPAR- γ is gender-dependently associated with cardio-metabolic risk factors.

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Beneficial effects and signalling pathways of AdipoR1/AdipoR2 activation in muscle like exercise and in liver on glucose/lipid metabolism and anti-inflammation

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Background and aims: The fat-derived hormone Adiponectin has been reported to produce a metabolic profile desirable for treating diseases of ageing such as type 2 diabetes and extend lifespan. In this study, we try to identify orally active AdipoR agonists.

Materials and methods: In skeletal muscle, by using muscle-specific AdipoR1 knockout mice, we showed that calcium signalling pathways as well as AMPK/SIRT1, and PGC-1 α are principal modulators of pathways downstream of Adiponectin/AdipoR1, which increase mitochondrial content and function, and ameliorate insulin resistance and glucose intolerance, like exercise. With regard to the liver, we show that hepatocyte-specific disruption of AdipoR1 results in increased molecules involved in gluconeogenesis, while hepatocyte-specific disruption of AdipoR2 results in decreased PPAR α pathways such as decreased molecules involved in fatty-acid combustion including ACO, both of which are associated with insulin resistance. We next try to identify and characterize orally active AdipoR agonists that are 1,000-fold more potent than adiponectin.

Results: One of these compounds, AdipoR agonist (ARA)-1 increases intracellular calcium concentration and PGC-1 α , and also activates AMPK in C2C12 myocytes. On a high-fat diet, ARA-1 activates AMPK and increases molecules involved in mitochondrial biogenesis such as PGC-1 α , Err α and Nrf1, molecules involved in fatty-acid combustion such as MCAD and oxidative stress-detoxifying enzymes in skeletal muscle, which are associated with increased insulin sensitivity, glucose tolerance and exercise endurance. In the liver, ARA-1 also activates AMPK and suppresses molecules involved in gluconeogenesis as well as activates PPAR α pathways such as increased molecules involved in fatty-acid combustion including ACO. In white adipose tissues, ARA-1 suppresses MCP-1. Importantly, in AdipoR1 and AdipoR2 double knockout mice, all these effects are almost completely abolished.

Conclusion: These data suggest that ARA-1 may activate both AdipoR1 and AdipoR2, and also that orally active AdipoR agonists are promising new therapeutic approach for treating diseases of ageing such as type 2 diabetes as exercise-mimetics.

PS 059 Inflammation and oxidative stress

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Aspirin inhibition of thromboxane does not affect oxidative stress, nitric oxide metabolites, paraoxonase activity or P-selectin levels in diabetesL.R. Lopez¹, J.R. Batuca², I.J. Muncy¹, I. Garcia De La Torre³, E. Matsuura⁴, P.R.J. Ames⁵;

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Background and aims: Aspirin (ASA) irreversibly inhibits platelet cyclooxygenase, leading to decreased thromboxane-mediated platelet activation/aggregation. We evaluated additional effects of ASA treatment (100mg/day x 7days) on oxidative stress, nitric oxide metabolites, P-Selectin, paraoxonase 1 (PON1) activity and anti-HDL antibodies in diabetes mellitus (DM).

Materials and methods: Serum and urine were obtained before ASA ingestion (baseline) and after 7 days (post-ASA) from 73 DM (mean age 54.3 years, mean disease duration 9.6 years) and 86 age/sex matched healthy controls. Urinary 11dhTxB2 and 8-isoPGF2 α , and serum P-Selectin, IgG anti-HDL antibodies were measured by ELISA. Serum nitrite (NO₂⁻) and nitrate (NO₃⁻) by modified Griess reaction and PON1 activity by para-nitrophenol formation.

Results: Compared to baseline controls, baseline DM had higher mean levels of 11dhTxB2 (3,665 \pm 2,465 vs 2,450 \pm 1,572 pg/mg creatinine, $p=0.002$), 8-isoPGF2 α (1,457 \pm 543 vs 1,009 \pm 412 pg/mg creatinine, $p<0.0001$), NO₂⁻ (11.8 \pm 7.3 vs 4.8 \pm 5.3 μ M, $p<0.0001$), NO₃⁻ (50.4 \pm 39.3 vs 20.9 \pm 16.7 μ M, $p<0.0001$) and P-Selectin (120.8 \pm 56.7 vs 93.0 \pm 26.1 ng/mL, $p=0.02$). Post ASA inhibition of 11dhTxB2 was 71.5% (3,665 to 996) in DM and 75.1% (2,450 to 624) in controls, but DM had twice as many ASA non-11dhTxB2 responders (>1,500) than controls (14.8% and 8.4%). Post-ASA 11dhTxB2 levels in DM (996 \pm 845) were significantly higher than controls (624 \pm 509, $p<0.0001$). ASA had no effect on 8-isoPGF2 α , NO₂⁻, NO₃⁻, P-Selectin, anti-HDL antibodies or PON1 activity in DM or controls.

Conclusion: ASA had no significant effect on oxidative stress, HDL-associated antioxidant PON1 activity, nitric oxide metabolites or P-Selectin in either DM or controls. The degree of 11dhTxB2 inhibition was similar in DM and controls though ASA non-responders were more frequent in DM despite greater baseline 11dhTxB2 concentration.

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The role of oxidative stress and inflammation on cellular senescence in diabetesS. Del Guerra¹, M. Gabriele¹, R. Lupi², F. Pancani¹, R. Pandolfi¹, S. Del Prato¹;

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Background and aims: A growing number of observations suggest that telomere shortening is associated with the spectrum of alterations of glucose metabolism. Telomere shortening characterizes both type 1 and type 2 diabetes, but initial telomere shortening has been reported in subjects with glucose intolerance as well. However, it is still unclear to which extent acceleration of telomere attrition and subsequent shortening is the consequence of hyperglycemia. In this study we have evaluated whether oxidative stress and chronic inflammation observed in diabetic patients can be associated with telomere length and telomerase activity.

Materials and methods: We recruited 28 non-diabetic subjects (ND: 49 \pm 10 yrs old; 16M/12F; BMI 24.6 \pm 3.3 Kg/m²), 19 type 1 diabetes (T1DM: 47 \pm 11 yrs; 8M/11F; BMI 25.5 \pm 2.2 Kg/m², Diabetes duration 26.6 \pm 10.3 yrs, HbA1c 8.4 \pm 0.3%) and 19 type 2 diabetes (T2DM age, 54 \pm 9 yrs; gender, 10M/9F; BMI 26.7 \pm 1.8 Kg/m², Diabetes duration 14.2 \pm 9.4 yrs, HbA1c 7.4 \pm 0.3%) patients. In all patients anthropometric data were collected and blood was drawn for lab tests and determination of nitrotyrosine (a marker of oxidative stress) concentration. Genomic DNA and total RNA were extracted from circulating nucleated blood cells. Telomere length was determined by Real-Time qPCR, while Real-Time RT-PCR was used for assessment of telomerase subunits

HTERT (catalytic component) and HTERC (RNA component), and CCR2 gene expression.

Results: As compared to ND (0.153 ± 0.025), telomere length was significantly reduced in T2DM (0.043 ± 0.011 ; $p < 0.001$) but not in T1DM (0.189 ± 0.077). On the contrary, the expression of HTERT ($+319 \pm 25\%$) but not HTERC subunits ($126 \pm 4\%$) was higher ($p < 0.05$) in T1DM than T2DM ($122 \pm 13\%$ and $147 \pm 5\%$, respectively). HTERT mRNA expression was higher in diabetics than in controls respect to ND (all $p < 0.05$ or less). After normalization by telomerase activity, a significant reduction in telomere length became apparent in T1DM (51.35 ± 4.7) and, to a greater extent, in T2DM (8.08 ± 1.45) as compared to ND (248.9 ± 44.6 ; all $p < 0.01$). In T1DM, but not in T2DM, the female gender was associated with a shorter telomere length. Telomere length was negatively associated with age in ND, while in T2DM was negatively related to age, total and LDL cholesterol and positively correlated with HDL-cholesterol. In T1DM it was inversely correlated with total while the relationship was positive with HDL-cholesterol concentration. CCR2 mRNA expression was higher in both T1DM ($+256.2 \pm 14.9\%$) and T2DM ($+656.9 \pm 58.4\%$) as compared to ND (all $p < 0.001$). In the study population as a whole, CCR2 mRNA expression was inversely correlated with telomere length ($r = 0.515$, $p = 0.039$) and positively correlated with HTERT ($r = 0.849$, $p < 0.001$) and HTERC ($r = 0.686$, $p = 0.019$). Similarly, nitrotyrosine levels were greater in diabetics (T2DM: 0.76 ± 0.09 OD, T1DM: 0.52 ± 0.07 OD), than in ND (0.31 ± 0.02 OD, all $p < 0.05$ or less). Moreover, a positive correlation was found between nitrotyrosine levels and CCR2 mRNA expression ($r = 0.513$, $p = 0.041$) and a negative one with telomere length ($r = 0.330$, $p = 0.038$) and C-peptide concentrations ($r = 0.281$, $p = 0.023$).

Conclusions: Our results confirm a link between telomere length, impaired telomerase activity and diabetes. We suggest that oxidative stress and inflammatory status can contribute to shortening of telomere in diabetic individuals.

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Regulation of the G6pc promoter activity by glucotoxicity: identification of a new mechanism involving ROS and the HIF-1 α transcriptional activity

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Background and aims: The activation of Glucose-6-phosphatase, which is the key enzyme of endogenous glucose production (EGP), is associated to type 2 diabetes. The expression of its catalytic unit (G6PC) is paradoxically increased by high glucose levels. This increase might thus contribute to unrestrained EGP characterizing this pathology. Glucotoxicity of type 2 diabetes is linked to the production of reactive oxygen species (ROS). ROS can induce gene expression by the induction of O-linked N-acetyl-glucosaminylation of transcription factors. ROS might also control gene expression through the regulation of the hypoxia inducible factor 1 (HIF-1). HIF-1 is composed of the HIF-1 α and HIF-1 β subunits. However, HIF-1 α only is regulated by hypoxia. In normoxia, the hydroxylation of HIF-1 α first induces its degradation and second inhibits its interaction with the cofactor CREB binding protein (CBP). Hydroxylation of HIF-1 α is inhibited by low O_2 levels or different stimuli such as ROS. In this work, we made the hypothesis that ROS induced by high glucose levels might control the transcription of G6pc through HIF-1 α .

Materials and methods: We analyzed the mechanism of the transcriptional control of G6pc by glucose in the hepatoma cell line HepG2. Cells were treated for 24 hrs with 5 or 25 mM glucose. ROS levels were decreased by the addition of an antioxidant (5.10^{-4} mol/L Trolox). Luciferase assays and chromatin immunoprecipitation were used to decipher molecular mechanisms. We confirmed the existence of the mechanisms evidenced in HepG2 cells in primary rat hepatocytes and in primary hepatocytes of a model of diet induced obesity (mice fed on a high fat-high sucrose diet).

Results: In HepG2 cells, glucose increased G6pc promoter activity in parallel to ROS levels. The addition of antioxidant prevented both the induction of G6pc promoter activity and the increase of ROS levels. The induction of G6pc promoter activity by glucose was prevented by a HIF-1 α dominant negative or a RNAi targeting CBP. In primary hepatocytes, we confirmed that decreasing the amount of ROS by antioxidant decreased the induction of G6pc mRNA levels by glucose. Glucose induced the stabilization of HIF-1 α protein and its binding to the G6pc promoter. Moreover, glucose increased the association of HIF-1 α with CBP and the binding of CBP to the G6pc promoter. The binding of CBP induced by glucose was prevented in the presence of antioxidant. Finally, we showed that levels of HIF-1 α protein and its binding to the G6pc promoter increased in the liver of mice fed on a high fat-high sucrose diet.

Conclusion: In this study, we identify a new mechanism of gene regulation by glucose. We show that glucose induces the expression of G6pc by a mechanism depending on ROS. We propose that ROS controls the transcriptional activity of HIF-1 α as does hypoxia. ROS first induce the stabilization of HIF-1 α and then allow the interaction of HIF-1 α with CBP. This mechanism may contribute to the increase of EGP during type 2 diabetes.

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The endothelial nitric oxide synthase (eNOS) co-factor tetrahydrobiopterin (BH4) suppresses hepatic gluconeogenesis and lowers blood glucose levels

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Background and aims: The tetrahydrobiopterin (BH4), an essential cofactor for endothelial nitric oxide synthase (eNOS), is crucial for the synthesis of nitric oxide by eNOS. In our previous report, we demonstrated that intra-hepatocellular eNOS is required for activation of AMP-activated protein kinase (AMPK) and suppression of hepatic gluconeogenesis by metformin in liver. Those findings suggest that nitric oxide produced by eNOS plays an important role in hepatic glucose metabolism. The aim of the present study was to determine whether BH4 suppresses gluconeogenesis and increases AMPK activation in hepatocytes.

Materials and methods: Hepatocytes were isolated from male 8-week-old C57/BL6 mice by collagenase digestion. Primary hepatocytes were maintained in DMEM with 10% fetal bovine serum. Protein expressions of AMPK α and phospho-AMPK α were analyzed by Western blot. For gluconeogenesis measurements, hepatocytes were isolated after overnight fast and incubated for 60 minutes in glucose-free KRB buffer with 1 mmol/l pyruvate plus 10 mmol/l lactate. Glucose production was determined by glucose oxidase method. In addition, eNOS-deficient mice were used to confirm the requirement for intra-hepatocellular nitric oxide production for the effect of BH4 in suppression of hepatic gluconeogenesis. To determine whether BH4 lowers blood glucose levels *in vivo*, 20 mg/kg BH4 was injected intraperitoneally to streptozotocin induced diabetic wild-type and eNOS-deficient mice and to AKITA mice fasted for 16 hours. To assess hepatic gluconeogenesis *in vivo*, pyruvate tolerance tests were performed in wild-type and eNOS-deficient mice.

Results: One-hour exposure of 50 μ mol/l BH4 significantly suppressed hepatic gluconeogenesis (BH4, 72 ± 17 ; control, 101 ± 12 nmol/mg protein; $p < 0.001$) and activated AMPK in wild type mouse hepatocytes. These effects of BH4 did not appear in eNOS-deficient mouse hepatocytes. Sepiapterin, a BH4 precursor, also suppressed hepatic gluconeogenesis significantly and activated AMPK. Sepiapterin had no effect on hepatic gluconeogenesis or AMPK activation in eNOS-deficient mouse hepatocytes. Sodium nitroprusside, a nitric oxide donor, suppressed hepatic gluconeogenesis significantly and activated AMPK in both wild-type and eNOS-deficient mouse hepatocytes. Administration of 20 mg/kg BH4 lowered blood glucose levels significantly in diabetic wild-type mice and AKITA mice ($p < 0.05$), but not in diabetic eNOS-deficient mice. In pyruvate tolerance test, 20 mg/kg BH4 injected intraperitoneally to wild-type mice decreased significantly elevation of blood glucose levels compared with saline injection. However, in eNOS-deficient mice, no effects of BH4 were detected.

Conclusion: BH4 suppresses hepatic gluconeogenesis and activates AMPK and lowers blood glucose levels eNOS-dependently. Supplementation of BH4 or its precursor might represent a novel therapeutic approach in type 2 diabetes through improvement of hepatic glucose metabolism.

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iNOS modulates the NF κ B signalling pathway through a NO-dependent pathway in models of inflammation induced insulin resistance

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Background and aims: It has been previously demonstrated that nitric oxide (NO) and various reactive nitrogen species modulate the NF κ B signaling pathway in cancer development and apoptosis. However, until now, no studies have tested whether iNOS activation modulates the NF κ B signaling pathway in metabolic tissues in conditions of insulin resistance. We have previously shown that

hepatic iNOS plays an important role in lipid- and endotoxin-induced insulin resistance and that this was mediated by an iNOS-dependent tyrosine nitration of insulin signaling proteins including Akt. Here, we evaluated whether iNOS can modulate NFκB activation through a NO-dependent mechanism.

Materials and methods: We subjected 8–12 weeks old WT and iNOS-KO mice to an acute injection of either saline (SAL) or LPS (20 mg/kg, i.p.) to assess insulin sensitivity during a hyperinsulinemic-euglycemic clamp protocol. Furthermore, we induced iNOS in a rat hepatic cell line (Fao) using a cytokine mixture (TNFα, IFNγ, IL-1β) in the presence or absence of the iNOS inhibitor 1400W to block inflammatory NO production.

Results: During euglycemic-hyperinsulinemic clamps we observed a strong reduction of the glucose infusion rate after treatment with LPS, which was completely inhibited in mice lacking iNOS. This was associated with improved insulin-mediated suppression of hepatic glucose production in iNOS-KO mice and increased Akt activation. Furthermore, a significant increase of IKKβ phosphorylation and tyrosine nitration of IκBα was observed in the livers of LPS-treated WT mice indicating an increase in NFκB activity. These effects of LPS were blunted in livers of iNOS-KO mice, suggesting a possible feedforward mechanism of NFκB activation by iNOS in this metabolic tissue. In agreement with the in vivo data, IKK phosphorylation and NFκB transcriptional activity were increased in Fao hepatic cells treated with cytokines, but this was reduced by inhibition of iNOS activity using the selective iNOS inhibitor 1400W.

Conclusion: We conclude that iNOS may positively activate the NFκB pathway through a feedforward mechanism and thus contribute to exacerbate inflammation and thereby further impairing insulin signaling and hepatic insulin action in inflammatory settings.

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Effect of dietary macronutrients on oxidative stress, cardiovascular risk factors, and insulin sensitivity in obese, non-diabetic, premenopausal women

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Background and aims: Various dietary interventions are known to result in significant weight loss and reduction in cardiovascular risk factors (CVR), but advantages of these diets are not established. We hypothesized that high-carbohydrate (CHO) diet (HC) (55% CHO, 30% fat, 15% protein) results in greater triglycerides (TG), oxidative stress (DCF), and lipid peroxidation (MDA) than high-protein (HP) diet (30% protein, 30% fat, and 40% CHO).

Material and methods: The diets based on resting energy expenditures with 500 Kcal reductions were provided by pre-packaged food on a weekly basis. To date, 13 (8HP, 5HC) non-diabetic, obese, pre-menopausal women were studied in a randomized prospective protocol. DCF, MDA, adiponectin and E-selectin were analyzed by ELIZA and colorimetric microplate assays. HOMA-IR was determined by the homeostasis model assessment of insulin resistance. CVR consisted of BP, BMI, and TG.

Results: The data depict results at baseline and after 6 months of diet.

Parameters measured	HP Baseline P* 6 months	HC Baseline P* 6 months	p**
BMI (kg/m ²)	42 ± 7.0 0.01 38.8 ± 7.3	37.5 ± 0.02 35.3 ± 5.4	0.28
% weight loss	0.01 8.6 ± 1.7	0.01 7.2 ± 1.9	0.60
BP (sys/diast)	129/83 ± 4/3 0.01 119/74 ± 3/3	128/82 ± 3/3 0.01 120/75 ± 3/3	0.70
TG (mg/dl)	102 ± 14 0.01 85 ± 10	102 ± 13 0.02 94 ± 9	0.02
DCF (μM)	3.2 ± 0.2 0.01 2.4 ± 0.1	3.2 ± 0.2 0.04 2.9 ± 0.1	0.02
MDA (μM)	1.1 ± 0.08 0.02 0.7 ± 0.05	1.1 ± 0.09 0.04 0.9 ± 0.7	0.04
HOMA-IR	3.6 ± 0.9 0.01 2.7 ± 0.4	3.5 ± 0.8 0.03 2.9 ± 0.5	0.03
Adiponectin (μg/ml)	5.56 ± 0.08 0.01 5.84 ± 0.08	5.52 ± 0.07 0.11 5.3 ± 0.07	0.02
E-Selectin (ng/ml)	42.6 ± 1.3 0.02 37.4 ± 1.2	43.4 ± 1.4 0.07 39.7 ± 1.6	0.03
% Adherence	91.8 ± 5.1	92.2 ± 7.3	0.92

*Wilcoxon Sign Rank Test

**Wilcoxon Rank Sum Test

Conclusions: HP resulted in increased insulin sensitivity (HOMA-IR), reduction in oxidative stress, lipid peroxidation, and TG compared to the HC diet, with similar weight losses and dietary compliance in both diets.

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The relationship between coronary atherosclerosis, functional exercise capacity and activin A in patients with type 2 diabetes

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Background and aims: Despite intensified control of risk factors, silent coronary artery disease (CAD) is still a frequent complication of type 2 diabetes (T2D) and associated with cardiac events. Therefore, new, validated biomarkers are needed to identify patients with silent CAD to reduce the need for invasive testing. Activin A, a multifunctional cytokine member of the TGF-β superfamily, plays a role in inflammation, atherogenesis and glucose homeostasis. We hypothesized that activin A is related to the extent of coronary artery disease in T2D and may be used as a marker for silent CAD.

Materials and methods: The present study is a substudy of the Asker and Baerum Cardiovascular Diabetes (ABCD) study and comprised 133 patients (pts) aged 18–75 years with T2D for a cross-sectional investigation of the prevalence of CAD. Participants underwent clinical examination, stress electrocardiogram and ergospirometry with gas exchange, in addition to blood and urine sampling. Pts with ≥1 CV risk factor were voluntarily referred to invasive coronary angiography and echocardiography. Between-group comparisons were conducted with t-, Mann-Whitney U- or chi squared test.

Results: Serum samples of activin A were available from 102 pts (mean ± SD age: 59 ± 10 y, 28% female, BMI 30.3 ± 5.5 kg/m², diabetes duration 6.7 ± 6.7 years) where 87 were free from previous CAD. The mean activin A level was 0.71 ± 1.16 ng/ml with min and max values of 0.04 and 11.79 ng/ml. Activin A in the 73 pts with T2D with coronary angiography tended to be higher in pts with CAD (> 50% luminal stenosis of major arteries, n=19) versus those without (n=54), i.e., 0.72 ± 0.37 vs 0.58 ± 0.36 ng/ml, p=0.16. The median value of activin A (0.56 ng/ml) for the overall T2D group (n=102) was set as cut-off to explore the association of activin A to anatomical indices of CAD, functional parameters and CV risk factors (table). Pts were comparable for history of smoking, hypertension, CRP and lipid levels, but those with activin A > median were older (61 ± 11 vs 56 ± 9 y, p=0.016) and had lower eGFR (96 ± 19 vs 84 ± 16 ml/min · 1.73 m² [p<0.01]), reduced exercise capacity and had significantly more CAD (table).

Conclusion: These observations imply that, in T2D, activin A may be a useful surrogate marker to define CV risk, and may facilitate early identification of patients with subclinical CAD. We speculate that the increased levels of activin A may reflect the underlying chronic pathological processes.

Pts characteristics and angiographic and exercise parameters according to level of Activin A

	Activin A ≤ 0.56 ng/mL	Activin A > 0.56 ng/mL	p-value
CRP (mg/l)	1.5 (0.9,3.0)	1.8 (1.2,2.9)	0.431
HbA1c (%)	7.4 (6.3,8.0)	7.1 (6.5,8.2)	0.755
UKPDS score (%)	14.5 ± 8.4	20.8 ± 12.5	0.005
MVO2 (O ₂ /kg/min)	25.4 ± 6.4	21.4 ± 5.8	0.004
[n=82]			
Measured MET	7.2 ± 1.8	6.5 ± 1.7	0.053
[n=82]			
Left ventricular ejection fraction (%)	64.2 ± 6.7	63.1 ± 8.3	0.552
[n=72]			
Coronary angiography: Stenosis > 50% (n=73)	6 (17%)	13 (35%)	0.062
Coronary angiography: ≥ 2 vessel disease (n=73)	1 (3%)	10 (27%)	0.004
Coronary angiography: Extent score (n=73)	0.64 ± 1.05	1.51 ± 2.04	0.024
Coronary angiography: Total severity (n=73)	3.4 ± 3.1	7.1 ± 4.5	0.023

Abbreviations: CRP - C-reactive protein, MET - metabolic equivalent, UKPDS - United Kingdom Prospective Diabetes Study, MVO2 - max O₂ uptake

Clinical Trial Registration Number: NCT00133718

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Clinical and metabolic risk factor association with c-reactive protein (hCRP) in type 2 diabetic subjects: base line finding from BNDC trial
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Background and aims: The development and progression of diabetes and its complications are greatly affected by inflammatory mechanisms. The aim of this study was to investigate the association of inflammatory marker high sensitive CRP (hCRP) with a number of clinical and laboratory parameters monitored during regular follow-up of a diabetic patient.

Materials and methods: Here base line data from an ongoing trial on controlling blood pressure, nephropathy and dyslipidemia in type 2 diabetics is primarily analyzed. In this study both diabetic nephropathy (DN) and those without (DM) were included. Association of hCRP at different cut-off values with blood pressure (BP), urinary protein (UTP), serum creatinine (SCr) HbA1c, lipids (LDL) and serum albumin (Alb) was observed.

Results: Total 730 subjects were included. Serum hCRP level was elevated (>3mg/l) in 55% subjects. Association study showed hCRP had no relation with bmi, systolic & diastolic BP, UTP or SCr but a positive correlation with HbA1c ($p<0.01$) and negative with Alb ($p<0.01$). An HbA1c above and below 7% was seen in 59 vs. 41% of all subjects with hCRP of 4.2 ± 4.9 vs. 5.2 ± 6.1 , mg/l, ($p<0.03$); LDL above and below 100mg/dl was seen in 48 vs. 52% with hCRP of 4.1 ± 4.5 vs. 5.3 ± 6.8 , mg/l, ($p<0.01$) and serum albumin above and below 4.0 g/dl cut-off in 60 vs. 40% subjects with the hCRP of 4.5 ± 5.1 vs. 5.6 ± 6.5 , mg/l, ($p<0.001$). The CRP levels didn't vary significantly for bmi above and below 25kg/m^2 and BP>140/90 mmHg. In sub grouping 70% ($n=513$) belonged to DN and 30% ($n=217$) DM group. The percentage of DN vs. DM subjects for hCRP cut-offs at <3 mg/l (39 vs. 62 %), 3-10 mg/l (50 vs. 30%) and >10 mg/l (12 vs. 8 %), ($p<0.001$) respectively showed higher values were present in both groups with more in nephropathy. In DN group comparisons according to same 3 hCRP cut-offs showed similar Systolic BP ($137\pm21, 138\pm21$ and 132 ± 19 , mmHg, $p=NS$), diastolic BP ($78\pm10, 79\pm9$ and 78 ± 9 , mmHg, $p=NS$) and also in DM group Systolic BP ($119\pm13, 120\pm12$ and 118 ± 18 , mmHg, $p=NS$) and diastolic BP ($75\pm8, 77\pm7$ and 72 ± 8 , mmHg, $p=NS$) were unchanged indicating no association of CRP with blood pressure. Similarly for different levels of CRP the UTP levels in DN subjects didn't vary ($1.8\pm2.0, 1.8\pm1.7$ and 1.8 ± 1.9 , g/day; $p=NS$).

Conclusion: It may be concluded that serum CRP level is elevated in majority of diabetic subjects and this has no apparent influence on levels of blood pressure or proteinuria. It seems that raised CRP is more linked to poor diabetes control with dyslipidemia and hypoalbuminemia indicating a higher prevalence of malnutrition-inflammation- atherosclerosis syndrome.

Supported by: Aristopharma, Aventis & Drug International

PS 060 Antidiabetic agents and cancer

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Effects of glucose on insulin stimulated cell proliferation

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Background and aims: It is well established that type 2 diabetic patients have a higher risk of certain cancers. However, the underlying mechanisms are not clear and both hyperglycemia and hyperinsulinemia have been proposed as possible links. The latter has been suggested to be the more important factor due to epidemiological findings and the fact that certain cancer cells have a constitutively high level of glucose uptake and are able to fully satisfy their glucose requirements at normoglycemia. However, the different glucose transporters (GLUTs) have different Km values. Thus depending on the GLUT expression pattern, glucose concentration could have an impact on glucose uptake and cellular proliferation. We examined the insulin-stimulated proliferative response in the presence of low (5 mM) and high (25-33 mM) glucose levels in a panel of different cell lines.

Materials and methods: L6 rat myoblasts overexpressing IR-A (L6-hIR), human breast (MCF7) or colon (COLO205) adenocarcinoma cells, and rat hepatoma cells (H4IIE). Receptor expression patterns were evaluated by FACS, Western blot or ELISA with monoclonal antibodies against the IR or IGF-IR. Cell proliferation was measured as incorporation of radioactively labeled thymidine using either traditional filter plates or cytostar-TTM scintillation microtiter plates.

Results: Three out of four cell lines showed a robust insulin-stimulated mitogenic response. The most pronounced response was seen in L6-hIR cells, where thymidine incorporation was increased more than 15-fold (EC_{50} of 1.5 nM). MCF7 and COLO205 cells also responded to insulin (3-4-fold) with EC_{50} values in the low nanomolar range. The H4IIE cells responded weakly to insulin (<2-fold) and only at high insulin levels. When the insulin dose-response effects were studied in either low or high glucose concentrations, no effect of high glucose was found in L6-hIR or MCF7 cells. If anything, a small inhibitory effect at 25 mM glucose was seen for MCF7 cells. In contrast, high glucose levels strongly promoted insulin-mediated thymidine incorporation in colon (COLO205) and liver (H4IIE) cancer cells. In COLO205 cells, a shift in the dose-response curve was observed. Thus, more than a doubling in DNA synthesis was seen at 100 pM insulin, but no effect was observed at maximal insulin concentration. Also in the absence of insulin there was an increased (1.6-fold) thymidine incorporation in response to high glucose. This insulin-independent effect was even higher in H4IIE cells showing a 3-4-fold increase in thymidine incorporation in response to high glucose.

Conclusion: We have found a significant difference in the proliferative response to glucose across a panel of cell lines. The growth of H4IIE (liver) and COLO205 (colon) cancer cells appears to be sensitive to glucose levels, whereas MCF7 (breast) cancer cells and myoblasts overexpressing the IR are unresponsive to glucose. Interestingly, liver and colon cancers are among the more frequently seen in patients with type 2 diabetes. Thus, control of blood glucose levels could be important for prevention of some cancer forms.

	IR	IGFIR	IR:IGF-IR ratio	Effect of insulin	Effect of glucose
L6-hIR	204.000	90.000	2:1	+++	no effect
MCF7	4.000	37.000	1:9	++	no effect
COLO205	2.100	13.900	1:7	++	++
H4IIE			1:6	(+)	+++

Supported by: Novo Nordisk

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Insulin glargine does not exhibit carcinogenicity in rodents: review of safety studies

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Background and aims: Insulin glargine (GLA) was tested in life-time carcinogenicity studies in rats and mice, targeting the incidence of spontaneously

occurring tumors and development of rare tumors. The 1995–1997 studies were reassessed for their validity with regard to design, survival rates and incidence of neoplastic mammary lesions in female animals.

Materials and methods: In both 2-year studies 50 animals per sex and per group were used. Three dose levels of GLA were used (2, 5, and 12.5 IU/kg) along with saline control (SC), vehicle control (VC) and NPH insulin (NPH; 12.5 IU/kg in mice or 5 IU/kg in rats) groups. Animals were palpated for nodules monthly until 6 months of age and then every 2 weeks to study end. Rats found dead were autopsied the same day. Statistical analyses were performed for each type of mammary tumor using a modified Peto lifetime adjusted analysis and the Bieler-Williams poly-3-test.

Results: Survival rates by weeks 80 and 90 in female mice were 50 and 30% (2 IU/kg GLA), 62 and 42% (5 IU/kg GLA), 72 and 54% (12.5 IU/kg GLA), 64 and 32% (SC), 52 and 34% (VC) and 56 and 42% (NPH), respectively. In female rats, they were 72 and 56% (2 IU/kg GLA), 74 and 60% (5 IU/kg GLA), 60 and 36% (12.5 IU/kg GLA), 70 and 60% (SC), 74 and 60% (VC) and 72 and 66% (NPH), respectively. Mammary tumors in mice were adenocarcinoma. Incidence was 0 (2 IU/kg GLA), 0 (5 IU/kg GLA) and 2 (12.5 IU/kg GLA) vs. 2 (SC), 0 (VC) and 2 (NPH). Mammary tumors in rats were mainly fibroadenoma and adenocarcinoma. Incidence of fibroadenoma was 26 (2 IU/kg GLA), 22 (5 IU/kg GLA) and 15 (12.5 IU/kg GLA) vs. 26 (SC), 21 (VC) and 28 (NPH). Incidence of adenocarcinoma was 7 (2 IU/kg GLA), 8 (5 IU/kg GLA) and 7 (12.5 IU/kg GLA) vs. 9 (SC), 9 (VC) and 7 (NPH). Statistical analysis revealed no significant differences between GLA and controls, including NPH insulin, in either mice or rats.

Conclusion: Both studies met current design practices and fulfilled the current FDA requirements that enough animals lived long enough to provide adequate exposure to GLA and to be at risk of forming late-developing tumors. The negative carcinogenicity studies confirm the long-term safety of insulin glargine, as derived from 2-year rodent studies.

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Metformin monotherapy or its combination with other antidiabetic drugs reduces cancer risk in type 2 diabetes subjects

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Background and aims: *In vitro* studies show that metformin reduces cellular proliferation, colonies formation and arrests growth of breast, colon, and prostate cancer cell lines, regardless its metabolic effect through the activation of AMPK-kinase. In humans metformin is associated to a reduction of cancer risk if compared to other antidiabetic medicines. Nevertheless, few evidences are available about the effect on cancer risk when metformin is associated to other antidiabetic drugs, and these results are controversial. The aim of our study is to evaluate the cancer risk in subjects with type 2 diabetes (DM2) in relation to hypoglycaemic treatment. Study hypothesis is that metformin, even in association to other hypoglycaemic therapies, reduces cancer risk compared to therapeutic regimens that do not include metformin.

Materials and methods: This is a retrospective study concerning an ambulatory cohort of patients followed up for DM2 (n=427).

Results: DM2 subjects showed a higher crude prevalence of cancer compared to a control population (426 non-diabetic hypertensive subjects) (12.6% vs 18.5%, p=0.02). Adjusted for age, sex, BMI and smoking habit, the risk of cancer diagnosis remained higher in DM2 individuals respect to non-diabetic controls (OR 1.77, 95% CI: 1.18 to 2.36). The incidence of cancer diagnosis among diabetic subjects was higher (29.3%) among individuals who never took metformin compared to subjects that have taken only metformin (10.0%), but also in comparison with those who received metformin in combination with other hypoglycaemic agents (18.3%). This difference remained unchanged even after adjustment for confounding covariates such as age, sex, smoking and BMI. Thus, the new diagnosis of cancer is statistically higher in patients who have never taken metformin. In particular, this group has an almost threefold risk of a diagnosis of cancer (HR: 2.93, 95% CI: 1.43 to 6.01) than metformin monotherapy group and than subjects treated with metformin in association with other hypoglycaemic agents. This risk is independent from age, sex, smoking and BMI.

Conclusion: Type 2 diabetes is associated with an increased risk of developing cancer respect to non-diabetics by 77%. In DM2 individuals metformin can reduce the risk of cancer regardless of associated hypoglycaemic treatment.

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Metformin inhibits IGF1 induced proliferation and signalling in a Caveolin-1 dependent manner in NSCLC cells

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Background and aims: The biguanide Metformin has potential antineoplastic activity. Metformin regulates cellular growth in cancer cells acting through cellular energy sensors with increase AMP/ATP ratio and activation of AMPK. We examined whether Metformin inhibits IGF1 induced proliferation and transduction pathway in Non Small Cell Lung Cancer (NSCLC) cell lines. Since Caveolin-1 expression plays a role in the cellular response to growth factor, we examined the effect of Metformin in Calu-1 and Calu-6: two NSCLC cell lines that respectively express high and low amount of Caveolin-1 (an essential component of caveolae).

Materials and methods: Biochemical analysis in Calu-1 and Calu-6 following Metformin and IGF1 treatment was performed by Western Blot. To modulate Cav-1 expression, Calu-1 and Calu-6 cells were transiently transfected respectively with a Cav-1 siRNA or Ctr siRNA and a pEGFPN1-plasmid expressing wt-Cav-1 or the empty vector. AMP/ATP ratio was measured by enzymatic assay.

Results: We observed that Calu-6 cells did not respond to acute Metformin inhibitory effect on IGF-IR induced cell growth and signalling, while Calu-1 cells were sensitive. Metformin inhibited IGF-I-induced proliferation of NSCLC in Calu-1 and resulted in a decrease phosphorylation of Akt and FOXO3a, a downstream target of Akt. Phosphorylation of FOXO3a by Akt induces its cytoplasmic sequestration and a reduced Akt signalling causes relocation of FOXO3a to the nucleus. In the presence of IGF1, Metformin retained Foxo3a into the nucleus in Calu-1 but not in Calu-6. We found that modulation of Cav-1 expression influenced Metformin action affecting AMPK phosphorylation as well as AMP/ATP ratio induced by Metformin. Silencing of Cav-1 in Calu-1 cells inhibited AMPK phosphorylation and AMP/ATP ratio increase induced by Metformin while Cav-1 expression in Calu-6 improved AMPK phosphorylation and AMP/ATP ratio increase in presence of Metformin.

Conclusion: Altogether, these data demonstrate that Metformin by AMPK phosphorylation and change of energy balance could inhibit IGF-IR signalling and raise the possibility of future utilization of AMPK activators in the treatment of NSCLC.

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Metformin inhibits insulin activation of the AKT and mTOR pathways and cellular proliferation in primary endometrial epithelial cells

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Background and aims: Obesity and type 2 diabetes are independent risk factors for the development of endometrial hyperplasia and carcinoma. Hyperinsulinemia is a hallmark of obesity and type 2 diabetes, and may play a role in endometrial cell proliferation. In several case reports, metformin has reversed progesterin-resistant endometrial hyperplasia. We sought to determine the effect of insulin on signaling pathways associated with hyperplasia in primary endometrial epithelial cells.

Materials and methods: Epithelial cells were separated from the endometrial tissues of ten women, and cultured in low glucose DMEM. Western blot analysis of phosphorylated and total AKT, 4E-BP1, and p70S6K was done after acute insulin stimulation in a dose range of 0.1 to 100nM for each group of epithelial cells. We then examined the relative concentrations of the same signaling proteins in cells pre-treated with 1 to 10mM of metformin, with and without insulin.

Results: Primary epithelial cells demonstrated a four fold increase (p=0.02) in cell proliferation over 24 hours in the presence of insulin relative to vehicle. Treatment with metformin abolished the proliferative effect of insulin. Insulin induced a robust dose-dependent phosphorylation of AKT and weak to robust activation of mTOR signaling in all ten primary epithelial cell cultures. This activation was inhibited by wortmannin, a PI3 kinase inhibitor. Metformin treatment of epithelial cells for 24hrs reduced the insulin-induced

phosphorylation of AKT, p70S6K, and 4E-BP1 at physiological doses of insulin (0.1–0.5nM). However, metformin did not alter insulin-induced AKT or mTOR pathway activation at supraphysiological doses of insulin (10–100nM). **Conclusion:** We have demonstrated that primary endometrial epithelial cells are sensitive to insulin, and that insulin likely promotes cell proliferation through activation of the AKT and mTOR pathways. Our data also provides preliminary evidence that metformin may have a direct effect on the endometrium, resulting in a reduced effect of insulin on cell proliferation and activation of signaling pathways. Metformin may be beneficial in the treatment of endometrial hyperplasia for women with hyperinsulinemia, in the setting of obesity and type 2 diabetes.

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Dipeptidyl peptidase-4 inhibitors and risk of cancer: myth or reality?

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Background and aims: Dipeptidyl peptidase-4 inhibitors (DPP4i) have been recently associated with increased risk of cancer and pancreatitis. Aim of the present meta-analysis of randomized clinical trials is the assessment of the effect of DPP4i on the incidence of cancer, and pancreatitis.

Materials and methods: A meta-analysis was performed including all randomized clinical trials with a duration of at least 24 weeks, enrolling patients with type 2 diabetes, comparing DPP4i with either placebo or active drugs.

Results: Fifty-three trials enrolling 20,312 and 13,569 patients for DPP4i and comparators, respectively, were included, reporting 176 malignancies and 22 pancreatitis. DPP4i, compared with placebo or other treatment, were associated with a similar risk of cancer (MH-OR 1.020[0.742–1.402]; $p=0.90$) and pancreatitis (0.786[0.357; 1.734], $p=0.55$).

Conclusion: The present meta-analysis seems to exclude any relevant short term effect of DPP4i on the incidence of cancer or pancreatitis.

PS 061 Diabetes and cancer

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Effect of different anatomical changes in gastrointestinal anatomy on glycaemic control after surgery in type 2 diabetic patients with early gastric cancer

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Background and aims: There is a growing body of evidence that remission of diabetes without medications, could be after bariatric surgery for morbid obese diabetic patients. Normal insulin levels and euglycemia are often observed within several days after surgery, suggesting that weight loss alone cannot explain why this surgery improves diabetes. Whereas diabetes is traditionally viewed as a chronic, relentless disease in which delay of end-organ complications is the major treatment goal, GI surgery may offer a novel end point. The role for GI surgery in diabetes treatment, however, is not clearly defined. To investigate whether diabetes control is the result of GI tract surgery which preserve duodenum or not, we have evaluated the glycemic conditions in the patients with diabetes mellitus who were treated for early stage of gastric cancer by Roux-en-Y method (not preserve duodenum) or Billroth I method (preserve duodenum).

Materials and methods: Using ICD10 codes, we identified patients undergoing Roux-en-Y gastrojejunostomy (RY) or Billroth I (BI) reconstruction over 1 year. Fasting blood glucose, total cholesterol, serum albumin, percentage of lymphocyte in white cell count and body weight before and after surgery were tabulated and compared using student's t-test. Oral hypoglycemic agent and pharmacological therapeutics were evaluated for the follow up period.

Results: Between 2008 and 2010, we identified 16 patients with T2DM out of cohort of 85 who underwent either RY (8 of 16) or BI reconstruction (8 of 16) for early gastric cancer and survived more than 30 days after operation. Study participants are all Japanese and 15 male and 1 female (RY M/F=8/0, BI M/F=7/1). Mean age of RY was 69.8 ± 7.0 and BI was 68.6 ± 9.6 years, HbA1c of RY was 6.8 ± 1.0 and BI was 6.4 ± 0.6 %. Average fasting plasma glucose of RY was 124.1 ± 30.7 , whereas that of BI was 119.4 ± 16.4 mg/dl. After 1 year follow up in RY HbA1c was 6.0 ± 0.8 % ($p < 0.05$, vs the value 1 year before) and BI was 6.3 ± 0.5 (ns, vs the value 1 year before). Body weight of both groups reduced similar, and serum albumin, T-cholesterol, percentage of lymphocyte levels were not changed in both groups. There was also no change in diabetes medication in both groups before and after surgery.

Conclusion: In the RY group the diabetic control was improved after one year follow up, independent of changes in body weight and diabetes medication. The reconstruction method may affect the glycemic control in diabetic patients.

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Mortality from common cancers among people with type 2 diabetes and the effect of socio-economic status

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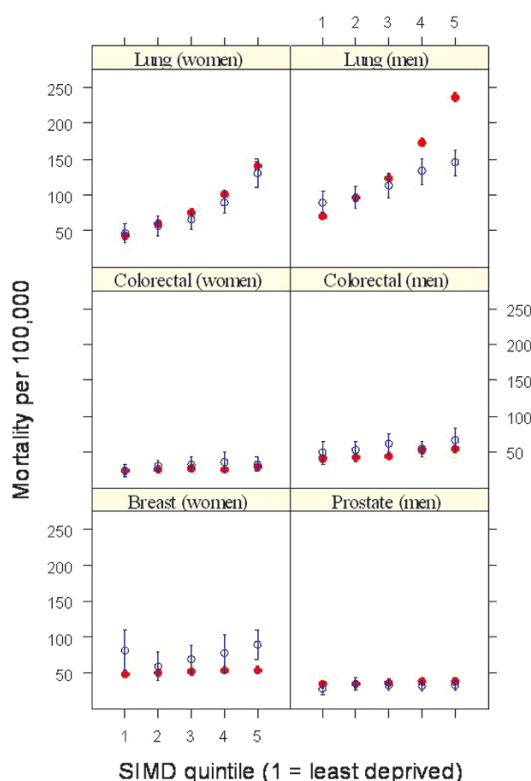
Background and aims: Type 2 diabetes (T2DM) is associated with increased incidence of several cancers, including those of the colon/rectum and breast and a protective effect against prostate cancer. Similar patterns for mortality from these common cancers might be expected although cardiovascular disease may act as a competing cause of death. The aims of this study were to describe absolute and relative mortality from common cancers (lung, colorectal, prostate and breast) among people with and without type 2 diabetes in the Scottish population and to investigate the effect of socio-economic status (SES).

Materials and methods: Data on people with diabetes were drawn from a population-based national diabetes register. Mortality rates for each cancer among men and women with T2DM who were aged 35–84 years in the period 2001–2007 were compared to corresponding rates for the non-diabetic Scottish population with direct age-standardisation using the European Standard Population. The non-diabetic population was created by subtracting numbers of deaths and person-years for people with type 1 or type 2 diabetes from the all-Scotland totals. SES was based on an area-based measure, the Scottish Index of Multiple Deprivation (SIMD), where Q1 and Q5

represent the most affluent and most deprived quintiles respectively. Poisson regression was used to estimate the relative risk (RR) of cancer mortality associated with T2DM compared to the non-diabetic population, adjusting for age or age and SES.

Results: Complete data were available for 210,994 people, representing 99.4% of those who had diabetes and were within the age range of interest for part or all of the study period. There were 2,081 deaths from lung cancer, 945 from colo-rectal cancer, 528 from breast cancer and 419 from prostate cancer. Age-adjusted mortality rates in both the T2DM cohort and general population, stratified by socio-economic status, are shown in the figure. Age adjusted RR (95% CI) for lung, colo-rectal and breast or prostate cancer were 0.94 (0.83 to 1.07), 1.18 (1.06 to 1.32), 1.32 (1.20 to 1.45) for women and 0.83 (0.73 to 0.93), 1.14 (1.04 to 1.25), 0.86 (0.78 to 0.95) for men. Age and SES adjusted RR (95% CI) for lung, colo-rectal and breast or prostate cancer were 0.89 (0.82 to 0.96), 1.17 (1.06 to 1.31), 1.31 (1.19 to 1.44) for women and 0.80 (0.75 to 0.86), 1.13 (1.04 to 1.24), 0.86 (0.78 to 0.95) for men.

Conclusion: There was a marked gradient by SES for lung cancer mortality for both people with and without T2DM with differing gradients for men with and without T2DM, possibly related to differing smoking habits and competing risks of mortality from cardiovascular disease. Compared to the non-diabetic population, T2DM was associated with higher mortality from colo-rectal cancer and breast cancer and lower mortality from lung and prostate cancer among men. Adjusting for SES had a small effect on overall RR for lung cancer but no effect for other cancers. There may be effect modification between T2DM and SES for lung cancer mortality among men.



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Retrospective evaluation of the safety of exogenous insulin in people with type 2 diabetes: cardiovascular events, stroke, cancers, all-cause mortality and a combined endpoint

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Background and aims: Recent observational and RCT data have raised concerns about the safety of treatment with exogenous insulin in people with type 2 diabetes. The purpose of this study was to compare outcome using alternative treatment regimens, and determine if this concern is warranted.

Materials and methods: Retrospective data were from UK general practices from 2000. The primary outcome was a combined endpoint of cardiovascular events, stroke, solid tumour cancers and all-cause mortality. Secondary endpoints were these constituent outcomes. Treatment cohorts were generated by characterising exposure to the following treatment regimens: metformin monotherapy vs. insulin-only regimens, insulin plus metformin combination therapies, sulfonylurea monotherapy, sulfonylurea plus metformin combination therapy, DPP-4s (monotherapy or in combination), and GLP-1s (monotherapy or in combination). Differences in baseline characteristics were accounted for using the Cox model (hazard ratio [HR] \pm 95%CI), with metformin monotherapy the referent.

Results: Cohort attribution was as follows: metformin, n=57,364 people (167,934 years follow-up); insulin-only 2,126 (9,268); insulin+metformin 2,158 (9,494); sulfonylurea 20,060 (64,193); sulfonylurea+metformin 16,464 (50,327); GLP-1s 757 (949); and DPP-4s 2,821 (3,125). The number of events was as follows: CV n=7,275, strokes 3,531, cancers 5,584, and deaths 8,424. The HRs are listed in the enclosed table following adjustment for potentially confounding characteristics.

Table. Adjusted hazard ratios for occurrence of diabetes-related endpoints by diabetes treatment.

	CV	Stroke	Cancer	Death	Combined
Insulin-only	1.85 (1.59-2.16)	1.69 (1.36-2.11)	1.63 (1.37-1.94)	2.54 (2.23-2.90)	1.99 (1.82-2.17)
Insulin+Met	1.54 (1.29-1.83)	1.02 (0.77-1.36)	1.47 (1.23-1.76)	1.61 (1.35-1.92)	1.52 (1.38-1.69)
Sulf	1.24 (1.13-1.35)	1.28 (1.16-1.42)	1.06 (0.97-1.16)	1.78 (1.66-1.90)	1.45 (1.39-1.51)
Sulf+Met	1.16 (1.06-1.27)	1.33 (1.19-1.48)	1.03 (0.94-1.12)	1.30 (1.20-1.41)	1.18 (1.12-1.23)
Metformin (ref)	1.00	1.00	1.00	1.00	1.00
GLP-1	0.84 (0.42-1.69)	1.95 (0.87-4.36)	1.11 (0.59-2.07)	1.05 (0.39-2.80)	0.85 (0.58-1.25)
DPP-4	0.91 (0.64-1.30)	1.31 (0.84-2.04)	0.83 (0.59-1.15)	0.82 (0.50-1.34)	0.74 (0.61-0.89)

Conclusion: Whilst accepting that a small group of patients require insulin replacement in type 2 diabetes, insulin-only regimens were consistently associated with the highest likelihood of progression to adverse outcomes. Together with biological plausibility and other intelligence, the safety and the role of exogenous insulin in type 2 diabetes is a concern and requires thorough scrutiny. Although there was relatively shorter follow-up, these data also demonstrated early promise for treatment with incretin-based therapies.

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Incidence and type of cancer in Japanese subjects with type 2 diabetes

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Background and aims: An association between diabetes and cancer has long been speculated, but no conclusive evidence has been obtained.

Materials and methods: We prospectively investigated the frequency and type of cancer in Japanese subjects with type 2 diabetes. Subjects included a total of 3136 consecutive patients with type 2 diabetes, who visited our clinic during 27 months from January 2009 to March 2011.

Results: Among 3136 subjects, a total of 64 patients (2.0%) were newly diagnosed to have cancer. The mean age was 70[10] years and male/female ratio was 41/22. The most frequent site of cancer was colon (n=14), followed by pancreas (n=12), stomach (n=12), liver (n=6), bile duct (n=4), lung (n=3), breast (n=3), kidney (n=3), esophagus (n=2), and others. The double cancer was found in two subjects. The A1C at the diagnosis of cancer was 8.3[2.4]%, and the therapy for diabetes was diet and exercise only (n=8), oral antidiabetic drugs (OADs) (n=34) and insulin (n=22). They included eleven patients, who had recently visited our clinic during these 6 months. The therapeutic variations (diet and exercise only 12.5%, OADs 53.1%, and insulin 34.3%) of the patients with cancer were not significantly different from those without malignancy in our clinic (diet and exercise only 18.0%, OADs 42.9%, and insulin 39.1%). The subjects without any symptom and/or sign were 54.2%.

In the cases with cancer who had recently visited our clinic during these 6 months ($n=11$), 50% of them were found to have pancreatic cancer. This is in line with a previous report, suggesting that onset of diabetes preceded the diagnosis of asymptomatic pancreatic cancer. The mean HbA1c was significantly higher in the patients with pancreatic cancer than that in the patients with other cancers (9.6 % vs. 7.4%, $p<0.05$). Furthermore, among the patients with pancreatic cancer, seven of eleven of them were found to have new-onset diabetes.

Conclusion: The present registry indicated that the incidence of cancer was considerably high in Japanese patients with type 2 diabetes, and that the therapeutic intervention, such as insulin or metformin, might not affect the incidence of cancer. Furthermore, nearly half of them were without any symptom and/or sign, justifying the routine screening for malignancy in subjects with type 2 diabetes.

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Limitations in the study of type 2 diabetes and pancreatic cancer in electronic insurance claims and health record databases

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Background and aims: While a higher incidence of pancreatic cancer (PC) has been associated with type 2 diabetes (T2D) in observational studies, the impact of reverse causality (impaired glucose metabolism as a consequence, rather than a cause, of PC) and tumor latency on this finding has not been adequately addressed. While electronic insurance claims and health record databases are valuable resources for the study of epidemiologic associations, the ability to consider reverse causality and latency is often limited. This study examined the impact of various latency periods on estimates of the incidence of PC in T2D and the association of T2D and subsequent PC in the General Practice Research Database (GPRD), an electronic health record database from the UK.

Materials and methods: A total of 3,127,581 patients (5.2% with T2D) were identified between 2003–2009 to assess the association of T2D and subsequent PC in patients diagnosed with T2D, relative to patients without T2D, using 1, 2, 3, and 5 year latency periods (time between T2D diagnosis or entry into study period and PC diagnoses). Patients with a history of PC were excluded. Multivariate Cox regression analyses adjusting for age, gender, prior history of chronic pancreatitis, gallbladder disease, obesity, smoking and alcohol use, and Charlson comorbidity index was used to estimate hazard ratios (HR) with 95% confidence intervals (CI).

Results: During this time, 1,934 patients were diagnosed with PC (442 with T2D, 1,492 without T2D). Patients with T2D consistently had a higher crude PC incidence compared to patients without T2D (table). Crude incidence increased with longer latency periods. Using all available data, crude incidence in patients with T2D was 78.8 per 100,000 vs. 11.6 per 100,000 in those without T2D. Using a 5 year latency period, crude incidence increased to 122.43 per 100,000 among patients with T2D and 25.89 per 100,000 in those without T2D. Adjusted HRs remained significant but point estimates attenuated slightly with longer latency periods. With a 5-year latency period, the adjusted HR was 1.5 (95% CI 1.06, 2.12), compared to a range from 1.80–1.84 with shorter or no latency periods.

Conclusion: Studies of the association of T2D and PC should account for reverse causality and latency to avoid underestimating PC incidence in patients with T2D and overestimating the HR. This is also relevant to studies of T2D treatments. These elements are often difficult to assess in electronic claims data due to the shorter duration of follow-up available. Electronic health record data can have the advantage of longer follow-up time and more complete data on potential confounders. However, even in an excellent electronic health record database such as GPRD, patient numbers decline quickly for rare conditions with long latency periods such as PC.

Latency Period	crude incidence rate per 100,000			
	Total cohort	T2D	Without T2D	Adjusted HR (95% CI)
Full cohort (n=1,934)	14.40	78.80 n=442	11.60 n=1,492	1.84 (1.56, 2.17)
1 year (n=1,653)	15.85	69.30 n=283	13.67 n=1,370	1.81 (1.52, 2.16)
2 years (n=1,285)	16.60	70.85 n=198	14.57 n=1,087	1.84 (1.51, 2.23)
3 years (n=953)	17.69	75.33 n=134	15.72 n=819	1.80 (1.43, 2.26)
5 years (n=440)	28.50	122.43 n=51	25.89 n=389	1.50 (1.06, 2.12)

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Synergistic effect of type 2 diabetes and history of chronic pancreatitis on pancreatic cancer risk: a retrospective cohort study from the General Practice Research Database (GPRD)

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Background and aims: The causes of pancreatic cancer (PC) remain largely unknown. Alcohol and tobacco use, gallbladder disease, and obesity are known risk factors. Many of these risks are also associated with chronic pancreatitis and type 2 diabetes (T2D). This study examined a potential synergistic association between T2D and chronic pancreatitis on PC risk in a retrospective cohort study in the GPRD.

Materials and methods: Data from 3,127,581 patients, 163,791 (5.2%) with T2D, identified between 2003–2009 were analyzed. Any past medical encounter for obesity (BMI \geq 30), chronic pancreatitis, or gallbladder disease occurring before a patient's index date were evaluated at baseline. Index date was the date of T2D diagnosis or date of entry into the study period for patients with existing T2D or no T2D. Patients with a history of PC were excluded. Multivariate Cox regression analysis covariates included Charlson comorbidity index and any known prior exposure to lifestyle risk factors such as tobacco and alcohol use. Crude and adjusted hazard ratios (HR) with 95% confidence intervals (CI) were calculated.

Results: During this time, 1,934 patients were diagnosed with PC (442 with T2D, 1,492 without T2D). T2D patients without a history of chronic pancreatitis had an elevated PC risk compared to patients without T2D and no history of chronic pancreatitis (adjusted HR = 3.00, 95%CI: 2.67, 3.37). Likewise, patients with chronic pancreatitis but without T2D had a 2-fold elevated risk, compared to those without either condition, but there were only 5 cases of PC in this group and the HR did not reach significance (HR: 2.3; 0.95, 5.56). Patients with T2D and a history of chronic pancreatitis had a much higher PC risk compared to patients without T2D and no history of chronic pancreatitis (adjusted HR = 14.96, 95%CI: 7.99, 28.02). Other risk factors associated with increased PC risk included age, alcohol use, and tobacco use.

Conclusion: After adjusting for known risk factors, T2D and chronic pancreatitis had a synergistic association on increased risk of PC compared to patients without T2D and without a history of chronic pancreatitis.

	N	Number of PC Cases	Adjusted HR	95% CI
Patients with no diabetes and no pancreatitis	2,960,982	1,487	1.00	
Patients with no diabetes and with pancreatitis	2,808	5	2.30	0.95, 5.56
Patients with T2D and no pancreatitis	162,741	432	3.00	2.67, 3.37
Patients with T2D and pancreatitis	1,050	10	14.96	7.99, 28.02

PS 062 Exenatide: twice daily to once weekly

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Exenatide twice daily: a retrospective analysis of pooled exenatide clinical trial efficacy data stratified by race, age, duration of diabetes, BMI and gender

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Background and aims: Many factors can influence the efficacy and safety of antidiabetic drug therapies including race, age, duration of diabetes, BMI, and gender. The purpose of this post hoc analysis was to examine endpoints, stratified by various subgroups, with the use of pooled clinical data from major exenatide BID (ExBID) trials.

Materials and methods: Patients included in this analysis had type 2 diabetes and received 10 µg ExBID in 12 comparator- or placebo-controlled studies. Mean changes from baseline, and corresponding 95% CI, were used to summarize efficacy endpoints for each group.

Results: A total of 1,877 patients were included (age [mean±SD] 57±10 y, HbA1c 8.3±1.0%, weight 93.0±19.3 kg; analysis endpoints at 24–30 weeks). [see table for results] Patients treated with ExBID experienced improvements in HbA1c, fasting glucose (FG), and weight loss from baseline, irrespective of age, duration of diabetes, or race. The reduction in FG was not as substantial in Blacks as it was in other racial groups; the lower baseline FG level in Blacks compared with that of other racial groups (8.2 mmol/L vs 9.2–9.9 mmol/L) may have contributed to this effect. Analyses of the BMI and gender subgroups showed similar efficacy results (data not shown). Overall, the most common adverse events were nausea (39.2%), vomiting (14.3%), and diarrhea (12.3%). Hypoglycaemia was more frequent in patients receiving sulphonylurea. Limitations of this post hoc analysis include a small number of patients in some groups, a lack of controls, and no adjustment for potentially confounding variables.

Conclusion: Patients treated with ExBID experienced improvements in HbA1c, FG, and weight loss from baseline, irrespective of age, duration of diabetes, race, BMI, or gender.

	Age		Duration of Diabetes		Race			
Selected	<65 years	≥65 years	<10 years	≥10 years	White	Black	Asian	Hispanic
Endpoints	(N=1,411)	(N=466)	(N=1,250)	(N=627)	(N=1,428)	(N=102)	(N=135)	(N=186)
HbA1c, mean (95% CI) Δ, %	-1.0 (-1.1, -0.9)	-1.1 (-1.2, -1.0)	-0.9 (-1.0, -0.9)	-1.2 (-1.2, -1.1)	-1.0 (-1.1, -0.9)	-0.8 (-1.1, -0.6)	-1.4 (-1.6, -1.2)	-0.9 (-1.1, -0.8)
Fasting glucose, mean (95% CI) Δ, mmol/L	-1.2 (-1.3, -1.0)	-0.9 (-1.2, -0.6)	-1.0 (-1.2, -0.8)	-1.3 (-1.6, -1.1)	-1.1 (-1.3, -1.0)	-0.1 (-0.6, 0.5)	-1.6 (-2.0, -1.2)	-1.0 (-1.6, -0.5)
Weight, mean (95% CI) Δ, kg	-2.4 (-2.6, -2.2)	-2.6 (-2.9, -2.3)	-2.5 (-2.7, -2.3)	-2.3 (-2.6, -2.0)	-2.6 (-2.8, -2.5)	-1.2 (-2.0, -0.5)	-1.8 (-2.4, -1.3)	-1.8 (-2.3, -1.3)
Systolic blood pressure, mean (95% CI) Δ, mmHg	-3.1 (-3.9, -2.3)	-4.5 (-6.0, -3.0)	-3.0 (-3.8, -2.2)	-4.3 (-5.6, -3.0)	-3.5 (-4.3, -2.7)	-5.7 (-9.2, -2.3)	-4.1 (-6.6, -1.6)	-2.2 (-4.6, 0.1)

Clinical Trial Registration Number: NCT00360334, NCT00375492, NCT00603239, NCT00765817, NCT00577824, NCT00434954

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DURATION-3: changes in cardiovascular risk factors observed in patients with type 2 diabetes after 84-week therapy with exenatide once weekly or insulin glargine

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Background and aims: In the DURATION-3 study, 456 patients with type 2 diabetes who did not achieve adequate glycaemic control on metformin alone or in combination with sulphonylurea were randomized to add exenatide once weekly (EQW) or insulin glargine once daily (IG). The majority of patients had abnormal lipids and/or BP at baseline. Patients in the EQW group experienced superior improvements in glycaemic control as measured by HbA1c compared to those in the IG group in the 26-week core study. EQW also was associated with significant weight loss as opposed to weight gain with IG. In post-hoc analyses, patients on EQW or IG experienced clinically significant changes in several surrogate markers of cardiovascular (CV) risk; those with abnormal baseline CV risk factors showed the greatest improvements. A study extension of up to 2.5 y was planned to evaluate sustained use of EQW in a controlled setting. Here we present interim data.

Materials and methods: Patients who entered the extension study remained on the initial therapy that they were assigned to for a total of up to 84 weeks. We repeated the post hoc analyses to assess changes in CV risk factors in patients with abnormal baseline values (defined in Table) and correlations between CV risk factors and body weight changes. We further analyzed 84-week data to determine whether patients achieved the following composite endpoints: Percentage of patients with HbA1c <7% (goal 1) or ≤6.5% (goal 2) plus SBP <130 mmHg and LDL <2.586 mmol/L.

Results: A total of 346 patients completed 84 weeks of therapy (EQW, 173; IG, 173). Patients with abnormal baseline values in both treatment groups continued to experience improvements in CV risk factors at week 84 (table). Body weight reductions also continued in the EQW group, while body weight continued to increase in the IG group: treatment difference -4.46 (0.30 kg) $P<0.001$. Body weight change for patients in the IG group was significantly correlated with change in SBP ($r^2=0.01736$, $P=0.0499$) and DBP ($r^2=0.02931$, $P=0.0106$). Significantly more patients in the EQW group achieved composite endpoint goal 1 (EQW: 15.7%, IG: 7.9%; $P=0.012$) and goal 2 (EQW: 11.2%, IG: 5.1%; $P=0.020$). The rate of new cases of adverse events declined after 26 weeks of treatment.

Conclusion: These data from patients receiving EQW for 84 weeks demonstrate its potential to be a long-term therapeutic option especially for the many patients with type 2 diabetes who fail to achieve blood glucose control while on oral agents and who have elevated CV risk factors.

Table. Mean Changes in Cardiovascular Risk Factors at Week 84

	SBP, mmHg	DBP, mmHg	LDL-C, mmol/L	HDL-C, mmol/L	TG, mmol/L	TC, mmol/L	hsCRP, mg/L
Abnormal threshold	≥130	≥80	≥2.6	<1.3/1.0†	≥1.7	≥5.2	>3
EQW: % Abnormal, BL/EP	66/52	58/52	51/46	47/46	54/51	34/31	45/36
EQW:	-7.69*	-4.99*	-0.22*	0.05*	-0.44*	-0.32*	-2.89*
Wk 84 Δ, Abnormal	(17.57)	(9.62)	(0.71)	(0.12)	(1.27)	(0.97)	(14.21)
EQW:	2.66*	3.31*	0.07	-0.03	0.23*	0.00	-0.05
Wk 84 Δ, Normal	(11.48)	(7.88)	(0.53)	(0.23)	(0.64)	(0.70)	(2.19)
IG: % Abnormal, BL/EP	61/61	61/55	51/52	46/47	53/45	34/29	45/43
IG:	-7.26*	-4.62*	-0.22*	0.01	-0.51*	-0.46*	-2.59*
Wk 84 Δ, Abnormal	(14.10)	(7.77)	(0.62)	(0.16)	(1.28)	(0.76)	(10.29)
IG:	9.35*	3.69*	0.13*	-0.04*	0.17	-0.01	0.76*
Wk 84 Δ, Normal	(12.32)	(8.93)	(0.55)	(0.17)	(1.05)	(0.71)	(3.04)

* $P<0.05$ vs. baseline; †Female/Male. All values shown are mean (SD) unless otherwise noted.

BL=baseline; EP= endpoint; SPB=systolic blood pressure; DBP=diastolic blood pressure; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; TG=triglycerides; TC=total cholesterol; hsCRP=high-sensitivity C-reactive protein.

Clinical Trial Registration Number: NCT00641056

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DURATION-4: Improvements in glucose control and cardiovascular risk factors in patients with type 2 diabetes treated with exenatide once weekly, metformin, pioglitazone, or sitagliptin

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Background and aims: Type 2 diabetic subjects have elevated cardiovascular (CV) risk factors including varying degrees of hypertension and dyslipidemia. We examined glycaemic control (primary), CV risk factors (secondary), and composite endpoint goals (exploratory) in drug-naïve subjects randomised to receive exenatide once weekly (EQW) vs. metformin (MET), pioglitazone (PIO), or sitagliptin (SITA) for 26 weeks.

Materials and methods: Patients in this double-blind study were randomised to subcutaneous (SC) EQW 2.0mg + oral placebo (PBO, N=248), MET + weekly SC PBO (N=246), PIO + weekly SC PBO (N=163), and SITA 100mg/d + weekly SC PBO (N=163). Baseline characteristics were: male, 59%; Caucasian, 67%; age, 54 y; HbA1c, 8.5%; fasting serum glucose (FSG), 9.9 mmol/L; body weight, 87.0 kg; and diabetes duration, 3.0 y (means). Changes in lipid-lowering and antihypertensive agents were only allowed if medically necessary. Post hoc analyses performed: 1) Change in CV risk factors (baseline abnormal); 2) Correlations between changes in risk factors and body weight for each treatment and; 3) Composite goals (Goal A - % of patients with HbA1c <7%, no weight gain, no minor or major hypoglycaemia at endpoint; Goal B - % with HbA1c <7%, systolic BP <130 mmHg, LDL <2.6 mmol/L at endpoint). **Results:** HbA1c reductions (%) at 26 weeks (least-squares [LS] means) with EQW vs. MET, PIO, and SITA were -1.53 vs. -1.48 (p=0.620), -1.63 (p=0.328), and -1.15 (p<0.001), respectively. LS mean changes in weight (kg) were -2.0 vs. -2.0 (p=0.892), +1.5 (p<0.001), and -0.8 (p<0.001), respectively. Except for total cholesterol, >50% of patients had abnormal lipids and blood pressure at baseline. Blood pressure and fasting lipids improved from baseline abnormal in all treatment groups (Table 1). Body weight change was not correlated to CV risk factors for EQW and PIO; weakly correlated with change in HDL (r²=0.04, p=0.003) and TG (r²=0.03, p=0.01) for MET; and weakly correlated with SBP (r²=0.04, p=0.01) and hsCRP (r²=0.04, p=0.03) for SITA. Composite goals were achieved most often with EQW and MET: 48% achieved Goal A for EQW vs. 46% (MET), 22% (PIO), and 35% (SITA); 16% achieved Goal B for EQW vs. 13%, 10%, and 7%.

Conclusion: Although all comparators improved glycaemic control at 26 weeks, variation was demonstrated with improvements in individual CV risk factors including body weight. EQW and MET were associated with greater achievement of composite goals of clinical relevance.

Table 1.

	EQW		MET		PIO		SITA	
	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
Systolic BP (mmHg)	138 (9)	-6 (13) ^a	140 (11)	-5 (14) ^a	143 (11)	-9 (14) ^a	139 (10)	-7 (11) ^a
Diastolic BP (mmHg)	85 (6)	-3 (8) ^a	85 (6)	-4 (7) ^a	86 (6)	-5 (9) ^a	85 (6)	-3 (7) ^a
LDL-C (mmol/L)	3.7 (0.9)	-0.4 (0.9) ^a	3.5 (0.7)	-0.3 (0.7) ^a	3.7 (0.8)	-0.1 (0.7)	3.6 (0.6)	-0.2 (0.6) ^a
HDL-C (mmol/L)	1.0 (0.2)	+0.1 (0.1) ^a	1.0 (0.2)	+0.1 (0.2) ^a	1.0 (0.2)	+0.2 (0.2) ^a	0.9 (0.2)	+0.1 (0.2) ^a
Triglycerides (mmol/L)	3.1 (2.0)	-0.4 (3.0)	2.9 (2.5)	-0.3 (2.6)	2.9 (1.5)	-0.7 (1.2) ^a	3.6 (4.1)	-0.8 (2.6) ^a
Total Cholesterol (mmol/L)	6.3 (0.9)	-0.6 (1.3) ^a	6.1 (0.8)	-0.5 (1.0) ^a	6.1 (0.9)	-0.1 (0.9)	6.1 (0.7)	-0.2 (0.8) ^a
hsCRP (mg/L)	9.6 (7.9)	-4.3 (8.5) ^a	8.5 (5.9)	-1.7 (7.1) ^a	8.5 (8.4)	-3.1 (8.2) ^a	8.6 (8.8)	+1.5 (16.5)

^ap<0.05 vs. baseline. Data are mean (SD). Baseline and change columns represent data from patients with abnormal values at baseline.

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Efficacy and safety of exenatide once weekly across background therapies: a pooled analysis of DURATION studies

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Background and aims: Treatment with the GLP-1 receptor agonist exenatide once weekly (QW) in patients with type 2 diabetes on a broad range of background therapies, ranging from diet and exercise to combination oral therapy, resulted in HbA1c reduction and weight loss (the DURATION clinical trials). It is of clinical interest to understand the safety and efficacy of exenatide QW across these background treatments.

Materials and methods: A pooled analysis by background therapy was performed on Intent-to-Treat (ITT) patients who initiated exenatide QW in the 24-30 week controlled periods of all four completed DURATION trials included in an ongoing integrated database (DURATION-1, -2, -3, and -5). For all efficacy endpoints, means and standard deviations were summarized for baseline values and means and 95% CI were reported for the change from baseline. Safety was reported as overall incidence.

Results: HbA1c and fasting glucose (FG) were significantly reduced with exenatide QW across all background therapies (Table). Weight loss was observed with exenatide QW in all cohorts, but did not reach statistical significance in the small cohort of patients on metformin (MET)+ thiazolidinedione (TZD) background. Nausea, the most frequent adverse event, occurred in 9% of patients who received exenatide QW monotherapy (diet/exercise) vs. 20%, 14%, and 35% for MET, MET+ sulphonylurea (SU), and MET+TZD background therapies, respectively. Nausea led to withdrawal in only 4 patients. Overall hypoglycaemia incidence (including unconfirmed symptoms of hypoglycaemia) was 33% in patients with MET+SU background vs. 7%, 8%, and 12% for diet/exercise, MET, and MET+TZD background therapies, respectively. One episode of hypoglycaemia necessitated the assistance of another person, but did not require medical intervention or involve loss or severe impairment of consciousness.

Conclusion: Patients treated with exenatide QW exhibited similar improvements in glycaemic control and body weight irrespective of background therapy.

Background Therapy	Diet/Exercise	MET	MET+TZD	MET+SU
DURATION Trial	1, 5	1, 2, 3, 5	1, 5	1, 3, 5
N	43	427	26	150
HbA1c Baseline (%)	8.4±1.1	8.4±1.1	8.1±1.0	8.5±1.1
Δ HbA1c (%)	-1.6 (-2.0, -1.2)	-1.4 (-1.6, -1.3)	-1.5 (-1.8, -1.2)	-1.5 (-1.6, -1.3)
HbA1c <7% at endpoint	72%	63%	65%	53%
HbA1c ≤6.5 % at endpoint	47%	42%	50%	39%
FG Baseline (mmol/L)	9.2±2.4	9.4±2.6	8.8±2.8	10.1±3.0
Δ FG (mmol/L)	-1.9 (-2.7, -1.1)	-1.9 (-2.2, -1.7)	-2.0 (-3.1, -1.0)	-2.0 (-2.5, -1.4)
FG ≤7 mmol/L at endpoint	56%	51%	58%	42%
Weight Baseline (kg)	97.0±20.6	92.7±19.4	103.1±21.9	94.4±19.7
Δ Weight (kg)	-2.3 (-3.5, -1.1)	-3.0 (-3.3, -2.6)	-2.3 (-4.6, 0.1)	-2.6 (-3.3, -1.9)

ITT per-patient analysis. Baseline = mean ± SD; Δ = mean (95%CI); MET = metformin.

Clinical Trial Registration Number: NCT00308139, NCT00637273, NCT00641056, NCT00877890

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Effects of exenatide once weekly on glycaemic goals and selected cardiovascular risk factors in patients with type 2 diabetes: a retrospective analysis of pooled clinical trial data

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Background and aims: Minimizing the most common cardiovascular and microvascular risks associated with type 2 diabetes (T2DM) is of primary concern in therapeutic decision making. Achieving early glycaemic control (HbA1c ≤6.5%) without hypoglycaemia has emerged as an important consideration. Beyond glycaemic control, therapeutic effects on established cardiovascular risk factors such as body weight, systolic blood pressure (SBP), and low-density lipoprotein (LDL) measurements are also clinically important. This retrospective pooled analysis from the DURATION clinical trial program for exenatide once weekly (ExQW) assessed the percentage of patients

achieving glycaemic goals. Additionally, changes from baseline in SBP and LDL were measured.

Materials and methods: The DURATION trials compared the efficacy (HbA1c) and safety of ExQW vs glucose-lowering comparators administered to patients for 24–30 weeks. Data from 804 patients who completed at least 20 weeks of ExQW exposure (defined as the completer population) were pooled and then stratified to identify patients who were not already at HbA1c goal at baseline (HbA1c >6.5%, $n = 800$; HbA1c $\geq 7\%$, $n = 774$). Patients were further stratified to isolate subpopulations of patients who were 1) not at recommended SBP measurements (SBP ≥ 130 mmHg) at baseline or 2) not at recommended LDL measurements (LDL ≥ 2.59 mmol/L) at baseline. The percentage of patients not already at goal who achieved glycaemic goals without hypoglycaemia or weight gain was assessed. Additionally, the mean changes in SBP and LDL from baseline to endpoint were assessed in the stratified populations.

Results: The baseline characteristics of the completer population were 45.0% women, mean age of 55 years, and a mean HbA1c of 8.4%. The results showed that 46.6% of patients achieved an HbA1c $\leq 6.5\%$ at endpoint and 64.6% of patients achieved an HbA1c <7% at endpoint. The percentage of patients able to achieve these goals without hypoglycaemia was 42.8% and 60.3% and without hypoglycaemia and weight gain was 36.8% and 50.1% for HbA1c $\leq 6.5\%$ and <7%, respectively. The mean \pm SEM change in HbA1c from baseline to endpoint in patients not already at HbA1c goal at baseline was $-1.52 \pm 0.04\%$ for the HbA1c $\leq 6.5\%$ goal and $-1.55 \pm 0.04\%$ for the HbA1c <7% goal. SBP and LDL improved in those patients not at the recommended goals for SBP or LDL at baseline. The mean \pm SEM change in SBP from baseline in the SBP ≥ 130 mmHg (at baseline) population was -7.41 ± 0.71 mmHg ($P < 0.0001$) and the mean \pm SEM change in LDL from baseline in the LDL ≥ 2.59 mmol/L (at baseline) population was -0.31 ± 0.04 mmol/L ($P < 0.0001$). No clinically significant changes in the mean SBP or LDL were noted in those patients treated with ExQW who were at the recommended SBP and LDL measurements at baseline. Adverse events in this analysis were consistent with those reported in the individual trials with nausea and diarrhea being most frequently reported.

Conclusion: This analysis demonstrated that in this population, treatment with ExQW significantly improved the percentage of patients achieving an HbA1c $\leq 6.5\%$ or <7% without causing hypoglycaemia or weight gain. Furthermore, in patients not at SBP or LDL goals, clinically meaningful improvements in SBP and LDL were observed.

Clinical Trial Registration Number: NCT00308139, NCT00637273, NCT00641056, NCT00676338, NCT00877890

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Exenatide once weekly: a retrospective analysis of pooled exenatide clinical trial efficacy data stratified by race, age, duration of diabetes, BMI and gender

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Background and aims: Many factors can influence the efficacy and safety of antihyperglycaemic drug therapies including race, age, duration of diabetes, BMI, and gender. The purpose of this post hoc analysis was to examine endpoints, stratified by various subgroups, with the use of pooled clinical trial data from major exenatide once weekly (ExQW) trials.

Materials and methods: Patients included in this analysis had type 2 diabetes and received exenatide in 4 comparator-controlled ExQW studies. Mean changes from baseline, and corresponding 95% CI, were used to summarize efficacy endpoints for each group.

Results: A total of 670 patients were included (age [mean \pm SD] 55 ± 10 y, HbA1c $8.4 \pm 1.1\%$, weight 94.1 ± 19.8 kg; analysis endpoints at 24–30 weeks). [see table for results] Patients treated with ExQW experienced improvements in HbA1c, fasting glucose (FG), and weight loss from baseline, irrespective of age, duration of diabetes, or race. Similar efficacy results emerged in the analyses of the BMI and gender subgroups (data not shown). Overall, the most common adverse events were nausea (18.8%), diarrhea (12.4%), and headache (8.2%). Minor hypoglycaemia occurred in 5% of patients; a single instance of hypoglycaemia occurred that necessitated the assistance of another person, but did not require medical intervention or involve loss or severe impairment of consciousness. Limitations of this post hoc analysis include a small number of patients in some groups, a lack of controls, and no adjustment for potentially confounding variables.

Conclusion: Patients treated with ExQW experienced improvements in HbA1c, FG, and weight loss from baseline, irrespective of age, duration of diabetes, race, BMI, or gender.

	Age		Duration of Diabetes		Race			
Selected Endpoints	<65 y (N=549)	≥ 65 y (N=121)	<10 years (N=496)	≥ 10 years (N=174)	White (N=447)	Black (N=36)	Asian (N=56)	Hispanic (N=131)
HbA1c, mean (95% CI) Δ , %	-1.5 (-1.6, -1.4)	-1.4 (-1.5, -1.2)	-1.4 (-1.5, -1.3)	-1.5 (-1.7, -1.4)	-1.4 (-1.5, -1.3)	-1.4 (-1.9, -0.9)	-1.5 (-1.8, -1.2)	-1.6 (-1.9, -1.4)
Fasting glucose, mean (95% CI) Δ , mmol/L	-1.8 (-2.1, -1.6)	-2.4 (-2.9, -2.0)	-1.8 (-2.1, -1.6)	-2.2 (-2.7, -1.7)	-2.0 (-2.2, -1.7)	-1.6 (-2.7, -0.5)	-1.8 (-2.7, -1.0)	-2.0 (-2.5, -1.4)
Weight, mean (95% CI) Δ , kg	-2.8 (-3.1, -2.4)	-2.8 (-3.3, -2.2)	-2.6 (-3.0, -2.3)	-3.2 (-3.9, -2.5)	-3.1 (-3.6, -2.7)	-2.0 (-3.5, -0.5)	-2.4 (-3.2, -1.5)	-1.9 (-2.5, -1.3)
Systolic blood pressure, mean (95% CI) Δ , mmHg	-3.0 (-4.2, -1.9)	-3.0 (-6.0, -0.1)	-2.9 (-4.0, -1.8)	-3.4 (-6.0, -0.8)	-3.7 (-5.1, -2.4)	0.2 (-4.6, 5.0)	-1.2 (-4.4, 2.1)	-2.3 (-4.5, -0.1)

Clinical Trial Registration Number: NCT00803920, NCT00637273, NCT00641056, NCT00877890

783

Exenatide once weekly: sustained improvement in glycaemic control and weight loss through 3 years

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Background and aims: In a 30-week controlled trial (DURATION-1), the once-weekly formulation of the GLP-1 receptor agonist exenatide (exenatide QW) elicited a more robust glucose lowering effect than the BID formulation of exenatide (ITT Δ HbA1c: -1.9% vs. -1.5%), coupled with similar weight loss, in patients with type 2 diabetes on a range of background therapies. This analysis was conducted to evaluate safety and efficacy following 3 years of treatment.

Materials and methods: The controlled period of the DURATION-1 trial was followed by an open-ended, open-label period in which all patients either continued exenatide QW treatment or switched from exenatide BID to exenatide QW. All efficacy endpoints were analyzed using the 3-year completer population. Means and standard deviations of the baseline values were summarized. Least square means and 95% confidence intervals based on general linear models were reported for the change from baseline. Adverse events are reported as overall incidence in the ITT population.

Results: Approximately 66% ($n=194$) of the 295 ITT patients completed 3 years of treatment (baseline [mean \pm SD]: HbA1c $8.2 \pm 1.0\%$; fasting plasma glucose (FPG) 9.3 ± 2.4 mmol/L; weight 101 ± 18 kg; duration of diabetes 7 ± 5 y; therapy at screening: diet/exercise [15%], metformin (MET) [33%], MET+sulphonylurea (SU) [29%], MET+thiazolidinedione (TZD) [9%]). Significant HbA1c improvement (LS mean [95%CI]) was observed with 3 years of treatment (-1.6% [-1.7, -1.4]), resulting in a mean \pm SEM HbA1c of $7.0 \pm 0.1\%$ (57% achieved HbA1c $\leq 7.0\%$ and 32% achieved an HbA1c $\leq 6.5\%$). Significant improvements in FPG (-1.8 mmol/L [-2.1, -1.5]); mean FPG at year 3: 7.3 ± 0.1 mmol/L and weight (-2.3kg [-3.4, -1.2]) were also observed. Furthermore, 3 years of treatment was associated with the following changes in cardiovascular risk markers: systolic blood pressure (-2.1 mmHg [-4.5, 0.2]), diastolic blood pressure (-2.0 mmHg [-3.3, -0.7]), total cholesterol (-0.26 mmol/L [-0.40, -0.11]), LDL cholesterol (-0.18 mmol/L [-0.31, -0.05]), and triglycerides (-12% [-18, -6]; geometric LS mean % change). Nausea (predominantly mild) was the most common adverse event with exenatide QW during the initial controlled period (27%), and decreased over time (16% from weeks 30–156). Injection-site pruritus and erythema were infrequent (<5%) from week 30–156 (vs 18% and 7%, respectively, in the 30-week controlled period). Treatment-emergent events leading to withdrawal from week 30–156 were also infrequent (3%), consistent with the 30-week controlled trial period (6%). As observed during the first 30 weeks of the trial, no major hypoglycaemia was observed in this open-label treatment period; incidence of minor hypoglycaemia was low in this treatment period, occurring predominantly with concomitant SU (16% incidence in patients on SU vs only 1% in patients not receiving concomitant SU; 15% and 0% incidence, respectively, during the controlled 30-week period). Correcting for cumulative exposure (patient-years), the adverse event incidence rates were lower in the open-label period when compared to rates observed in the initial controlled period.

Conclusion: Exenatide QW elicited a robust and sustained improvement in glycaemic control in patients with type 2 diabetes who were on an array of background treatments. Importantly, this effect was associated with improvements in broader cardiometabolic measures, including body weight and lipids.

Clinical Trial Registration Number: NCT00308139

PS 063 GLP-1 receptor agonists: new drugs and new formulations

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Efficacy and safety of lixisenatide once-daily versus placebo in patients with type 2 diabetes mellitus insufficiently controlled on metformin (GetGoal-F1)

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Background and aims: This randomised, double-blind, placebo-controlled, parallel-group, multicentre study compared the efficacy and safety of lixisenatide QD in two dose-increase regimens vs placebo as add-on therapy in T2DM inadequately controlled by metformin.

Materials and methods: Patients with T2DM (n=482) treated with metformin (mean age 56.1 yr, diabetes duration 6.0 yr, BMI 32.5 kg/m², HbA_{1c} 8.0%) were randomised to one of 4 QD regimens: lixisenatide 2-step dose increase (10 µg for 1 wk, 15 µg for 1 wk then 20 µg; n=161), lixisenatide 1-step dose increase (10 µg for 2 wk then 20 µg; n=161), placebo 2-step (n=79) or placebo 1-step (n=81) (placebo groups were combined). After screening of up to 3 wk, patients entered a 24-wk main treatment period, followed by a variable extension of at least 52 wk, with lifestyle advice every 3 mo. The primary objective was to assess the efficacy of lixisenatide (2-step regimen) vs placebo in terms of HbA_{1c} reduction at Wk 24. Efficacy of the 1-step regimen was the next test in a step-down testing procedure.

Results: The 24-wk main treatment period data are presented. There were significant improvements in HbA_{1c} from baseline to Wk 24 in both lixisenatide groups vs placebo (p<0.0001) (Table). Significantly more patients in the lixisenatide groups achieved HbA_{1c} ≤6.5% (20.4% 2-step, 25.6% 1-step) and <7.0% (42.1% 2-step, 47.4% 1-step) vs placebo (7.6% and 24.1%, respectively; p<0.001). There were also significant improvements in FPG and body weight with lixisenatide vs placebo (Table). Rates of premature treatment discontinuation at Wk 24 were 10.6% in the lixisenatide 2-step, 8.7% in the 1-step and 5.6% in the placebo groups. Fewer patients required rescue therapy in both lixisenatide groups than in the placebo group (3.1% [2-step] and 1.3% [1-step] vs 4.4%). The incidence of patients with serious AEs was comparable in the lixisenatide groups (4.3% [2-step]; 3.1% [1-step]) and the placebo group (2.5%). The overall incidence of patients with AEs was 70.8% for lixisenatide 2-step, 67.7% for 1-step and 65.6% for placebo. The most frequently reported AEs were nausea (35.4% [2-step] and 26.1% [1-step] vs 0% with placebo) and vomiting (15.5% [2-step] and 11.8% [1-step] vs 0% with placebo). No significant difference was seen in the % of patients with symptomatic hypoglycaemia between the lixisenatide groups (2.5% [2-step]; 1.9% [1-step]) and placebo (0.6%; p>0.1). There were no cases of severe hypoglycaemia.

Conclusion: Lixisenatide QD administered in a 1- or 2- dose increase regimen significantly improved glycaemic control and decreased weight over 24 wks with no increased risk of hypoglycaemia in T2DM patients insufficiently controlled on metformin.

Parameter	Lixisenatide 2-step dose increase	Lixisenatide 1-step dose increase	Placebo
Mean baseline and 24-week changes in efficacy parameters (mITT population)	N=160	N=158	N=159
HbA _{1c} (%)	Baseline±SD	Baseline±SD	Baseline±SD
LS mean±SE change from baseline	8.12±0.89	7.99±0.88	8.03±0.83
LS mean difference vs placebo [95% CI]; p-value	-0.83±0.10	-0.92±0.10	-0.42±0.10
LS mean difference vs placebo [95% CI]; p-value	-0.41 [-0.58 to -0.23]; p<0.0001	-0.49 [-0.67 to -0.32]; p<0.0001	—
Fasting plasma glucose (mmol/L)	Baseline±SD	Baseline±SD	Baseline±SD
LS mean±SE change from baseline	9.52±2.50	9.55±2.04	9.46±1.95
LS mean difference vs placebo [95% CI]; p-value	-0.56±0.21	-0.53±0.21	+0.11±0.21
LS mean difference vs placebo [95% CI]; p-value	-0.67 [-1.04 to -0.30]; p=0.0004	-0.65 [-1.02 to -0.28]; p=0.0007	—
Body weight (kg)	Baseline±SD	Baseline±SD	Baseline±SD
LS mean±SE change from baseline	88.1±16.8	90.3±19.0	87.9±17.3
LS mean difference vs placebo [95% CI]; p-value	-2.68±0.39	-2.63±0.39	-1.63±0.39
LS mean difference vs placebo [95% CI]; p-value	-1.05 [-1.73 to -0.37]; p=0.0025	-1.00 [-1.69 to -0.32]; p=0.0042	—
Safety parameters (safety population)	N=161	N=161	N=160
N (%) of patients with symptomatic hypoglycaemia*	4 (2.5%)	3 (1.9%)	1 (0.6%) p>0.1

*event with clinical symptoms with either plasma glucose <3.3 mmol/L or prompt recovery after oral carbohydrate administration if no plasma glucose measurement was available.

Clinical Trial Registration Number: NCT00763451

Supported by: sanofi aventis

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Efficacy and safety of lixisenatide once-daily versus placebo in patients with type 2 diabetes mellitus insufficiently controlled on sulfonylurea ± metformin (GetGoal-S)

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Background and aims: This randomised, double-blind, placebo-controlled, two-arm, parallel-group, multicentre study compared the efficacy and safety of once-daily (QD) lixisenatide vs placebo as add-on therapy in people with T2DM inadequately controlled by sulfonylurea ± metformin.

Materials and methods: A total of 859 patients with T2DM treated with a sulfonylurea ± metformin (16% on sulfonylurea alone, mean age 57.2 yrs, diabetes duration 9.4 yrs, BMI 30.2 kg/m², baseline HbA_{1c} 8.3%) were randomised to lixisenatide 20 µg QD (n=573) or placebo (n=286) in a 2-step dose increase regimen. After a screening period of up to 3 weeks, patients entered a 24-week main treatment period, followed by a variable extension of at least 52 weeks. The primary objective was to assess the efficacy of lixisenatide vs placebo in terms of HbA_{1c} reduction at Week 24.

Results: The 24-week main treatment period data are presented. Lixisenatide QD significantly reduced HbA_{1c} compared to placebo (-0.85 vs -0.10%; p<0.0001). Lixisenatide QD vs placebo also significantly improved 2-hour PPG (after a standardised meal test) (-6.19 vs -0.21 mmol/L), FPG (-0.99 vs -0.36 mmol/L) and body weight (-1.76 vs -0.93 kg) and increased the proportion of patients achieving HbA_{1c} <7.0% (36.4 vs 13.5%) (p<0.0001 for all; see Table). The percentage of patients requiring rescue therapy was significantly lower in the lixisenatide group than in the placebo group (3.5% and 12.0% respectively, p<0.0001). Rates of premature treatment discontinuation at Week 24 were 12.9% in the lixisenatide group and 10.8% in the placebo group. The overall incidence of patients with adverse events (AEs) was 68.3% in the lixisenatide group and 61.1% in the placebo group. The difference between groups was mainly attributable to GI events, mainly nausea (25.3% with lixisenatide vs 7.0% with placebo) and vomiting (8.7% vs 3.5%). No difference was seen in the incidence of patients with serious AEs between lixisenatide (3.5%) and placebo (5.6%). Lixisenatide QD did not significantly increase symptomatic hypoglycaemia vs placebo (Table). There was 1 case (0.2%) of severe hypoglycaemia in the lixisenatide group and none in the placebo group.

Conclusion: Add-on treatment with lixisenatide once-daily significantly improved glycaemic control with weight loss, without increasing the risk of hypoglycaemia, over 24 weeks in T2DM patients insufficiently controlled on sulfonylurea ± metformin.

Parameter	Lixisenatide	Placebo	LS mean difference [95% CI]; p-value
Mean baseline and 24-week changes in efficacy parameters (mITT population)	N=564	N=284	
HbA _{1c} (%)	Baseline±SD	Baseline±SD	Baseline±SD
LS mean±SE change from baseline	8.28±0.86	8.22±0.83	-0.74 [-0.87 to -0.62]; p<0.0001
2-hour postprandial plasma glucose (mmol/L)*	Baseline±SD	Baseline±SD	Baseline±SD
LS mean±SE change from baseline	16.61±4.09	16.55±3.74	-5.98 [-6.91 to -5.04]; p<0.0001
Fasting plasma glucose (mmol/L)	Baseline±SD	Baseline±SD	Baseline±SD
LS mean±SE change from baseline	9.67±2.24	9.29±2.37	-0.63 [-0.92 to -0.35]; p<0.0001
Body weight (kg)	Baseline±SD	Baseline±SD	Baseline±SD
LS mean±SE change from baseline	82.6±21.9	84.5±22.8	-0.84 [-1.25 to -0.42]; p<0.0001
Proportion achieving HbA _{1c} <7.0%	n (%)	n (%)	p<0.0001
	198 (36.4%)	37 (13.5%)	
Safety parameters (safety population)	N=574	N=285	p-value
N (%) of patients with symptomatic hypoglycaemia**	88 (15.3%)	35 (12.3%)	>0.1

*using a standardised meal-test in selected sites (lixisenatide: n=249, placebo: n=120)

**event with clinical symptoms with either plasma glucose <3.3 mmol/L or prompt recovery after oral carbohydrate administration if no plasma glucose measurement was available.

Clinical Trial Registration Number: NCT00713830

Supported by: sanofi-aventis

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Efficacy and safety of lixisenatide once-daily versus exenatide twice-daily in patients with type 2 diabetes insufficiently controlled on metformin (GetGoal-X)

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Background and aims: This randomised, open-label, active-controlled, parallel-group, multicentre study compared the efficacy and safety of lixisenatide QD vs exenatide BID as add-on therapy in T2DM inadequately controlled on metformin monotherapy.

Materials and methods: Individuals with T2DM (n=634) treated with metformin ≥1.5 g/day (mean age 57.4 yr, diabetes duration 6.8 yr, BMI 33.6 kg/m², HbA_{1c} 8.0%) were randomised to lixisenatide 20 µg QD (n=318) or exenatide 10 µg BID (n=316). After a screening period of up to 2 wk, subjects entered a 24-wk main treatment period, followed by a variable extension of at least 52 wk. Both groups received a stepwise increase in dose up to a maximum 20 µg/day. The primary objective was to demonstrate non-inferiority of lixisenatide to exenatide in terms of HbA_{1c} reduction at Wk 24. Non-inferiority was demonstrated if the upper limit of the 2-sided 95% CI of the difference between lixisenatide and exenatide in adjusted mean change in HbA_{1c} from baseline to Wk 24 was ≤0.4%.

Results: The 24-wk main treatment period data are presented. Lixisenatide QD achieved its primary endpoint of non-inferiority in HbA_{1c} reduction from baseline [BL] at Wk 24 vs exenatide BID (Table). Improvements in mean FPG and the proportion of subjects achieving HbA_{1c} <7.0% were comparable between groups (Table). Mean body weight significantly decreased from BL in both groups: 94.5 to 91.7 kg with lixisenatide and 96.7 to 92.9 kg with exenatide. The incidence of subjects with adverse events (AEs) was similar for lixisenatide (69.5%) and exenatide (72.2%). Rates of premature treatment discontinuation were 12.9% and 14.2% for lixisenatide and exenatide, respectively, and discontinuations due to AEs (mainly GI events) were 33 (10.4%) in lixisenatide and 41 (13.0%) in exenatide subjects. Serious AEs occurred in 9 lixisenatide (2.8%) and 7 exenatide (2.2%) subjects. Significantly fewer subjects experienced symptomatic hypoglycaemia with lixisenatide, with 6-fold fewer hypoglycaemic events with lixisenatide (Table). There were no cases of severe hypoglycaemia. Overall GI tolerability appeared better for lixisenatide vs exenatide, with fewer experiencing nausea and vomiting (Table). More subjects in the lixisenatide group tolerated the targeted maintenance dose of 20 µg/day (93% vs 83% exenatide).

Conclusion: Add-on lixisenatide QD in T2DM insufficiently controlled on metformin demonstrated non-inferior improvements in HbA_{1c}, but with less

hypoglycaemia, slightly less weight loss and a more favourable GI tolerability profile compared with exenatide BID at Wk 24.

Parameter		Lixisenatide	Exenatide	
Mean baseline and 24-week changes in efficacy parameters (mITT population)		N=311	N=305	LS mean difference [95% CI]; p-value
HbA _{1c} (%)	Baseline±SD	7.97±0.82	7.96±0.77	0.17 [0.03 to 0.30] (non-inferior based on upper limit of 95% CI ≤0.4)
	LS mean±SE change from baseline	-0.79 ±0.05	-0.96±0.05	
Fasting plasma glucose (mmol/L)	Baseline±SD	9.72±2.03	9.68±2.25	0.23 [-0.05 to 0.52]
	LS mean±SE change from baseline	-1.22±0.12	-1.45±0.12	
Body weight (kg)	Baseline±SD	94.5±19.4	96.7±22.8	1.02 [0.46 to 1.58]
	LS mean±SE change from baseline	-2.96±0.23	-3.98±0.23	
Proportion achieving HbA _{1c} <7.0%	n (%)	143 (48.5%)	148 (49.8%)	p=NS
Safety parameters (safety population)		N=318	N=316	p-value
N (%) of patients with symptomatic hypoglycaemia *		8 (2.5%)	25 (7.9%)	<0.05
N of hypoglycaemic events		8	48	
N (%) of patients with nausea		78 (24.5%)	111 (35.1%)	<0.05
N (%) of patients with diarrhoea		33 (10.4%)	42 (13.3%)	NS
N (%) of patients with vomiting		32 (10.1%)	42 (13.3%)	NS

*event with clinical symptoms with either plasma glucose <3.3 mmol/L or prompt recovery after oral carbohydrate administration if no plasma glucose measurement was available

Clinical Trial Registration Number: NCT00707031

Supported by: sanofi aventis

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A placebo controlled single ascending dose Phase 1 for safety, tolerability, pharmacokinetics and pharmacodynamics of VRS-859 in patients with T2DM

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Background and aims: VRS-859 is a novel fusion protein containing the GLP-1 analogue, exenatide, and a long hydrophilic tail of natural amino acids, XTEN, which increases the half-life. VRS-859 is being developed as a once monthly injection for the treatment of type 2 diabetes mellitus (T2DM).

Materials and methods: Up to 55 T2DM patients on metformin (met) or met and sulfonylurea (SU) are proposed for the study to evaluate the safety/tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of VRS-859. T2DM patients on met+SU are withdrawn from SU treatment 2 weeks prior to dosing and remain on met only. Each patient receives a single subcutaneous dose of VRS-859 or placebo on Day 1. Dose levels are 12.5, 25, 50, 100 and 150 mg VRS-859. In each treatment group, 8 patients receive a single dose level of VRS-859 and 3 patients receive placebo in a randomized blinded manner. PD measurements (fasting glucose (FG) and insulin and oral glucose tolerance test (oGTT)) are performed on Days -1, 4, 8, 11, 15, 18, 22, 25, and 30. Safety assessments including calcitonin, lipase, and amylase levels as well as QTc analyses are performed through Day 30. Anti-VRS-859 antibody analyses are performed at Day -1, Day 30 and on follow-up at Day 60.

Results: This clinical study is ongoing with patients currently enrolling in the 100 mg dose cohort, the dose expected to provide once monthly therapy. To date, 29 patients have been enrolled: 8 patients received 12.5 mg VRS-859, 8 patients received 25 mg VRS-859, 6 patients received 50 mg VRS-859, and 7 patients received placebo. In patients treated with VRS-859, no notable AEs and no changes in QTc have been observed. No anti-VRS-859 antibodies have been detected. The PK of VRS-859 indicated a T_{max} of approximately 3 days post-dose and a half-life of approximately 120 hrs. The mean reduction in FG from baseline at Day 4 and Day 8 for patients receiving 25 mg VRS-859 was 36.7 mg/dL and 25.9 mg/dL, respectively. The mean reduction in glucose AUC after oGTT compared to baseline at Day 4 and Day 8 for patients receiving 25 mg VRS-859 was 56.3 mg.hr/dL and 43.2 mg.hr/dL, respectively. These initial results suggest that 25 mg VRS-859 provides effective glycemic control over a week after dosing without gastrointestinal adverse events (a known side effect of this class of drug).

Conclusion: VRS-859 is well tolerated and provides glycemic control in T2DM patients. Additional results from enrolled dose levels including the anticipated monthly dose (100 mg) will be presented.

Clinical Trial Registration Number: Eudract number: 2010-019182-29

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Monotherapy with the once-weekly GLP-1 analogue LY2189265 for 12 weeks in patients with type 2 diabetes: dose-dependent effects in a randomised, double-blind, placebo-controlled study

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Background and aims: We evaluated the dose-dependent effects of once-weekly LY2189265 (LY), a glucagon-like peptide-1 (GLP-1) analog, on glycaemic control [hemoglobin A_{1c} (HbA_{1c})] in patients with type 2 diabetes treated with life-style measures.

Materials and methods: This was a double-blind, placebo-controlled study in 164 patients (at entry, mean±SD: age 57±9 years; BMI 32.1±4.8 kg/m²; and HbA_{1c} 7.2±0.6%) who were antihyperglycaemic medication naïve or had discontinued metformin monotherapy. Patients entered a 4 to 8-week lead-in period. Those with qualifying HbA_{1c} values (≥6.5% to ≤9.5%) were randomized (mean baseline HbA_{1c} between 7.6±0.7% and 7.8±0.8%) to once-weekly subcutaneous injections of placebo or LY (0.1 mg, 0.5 mg, 1.0 mg, or 1.5 mg) for 12 weeks, followed by a 4-week safety follow up.

Results: At week 12, statistically significant dose-dependent reductions in HbA_{1c} (LS mean±SE) were observed across LY2189265 doses (p<0.001). HbA_{1c} reductions in LY0.5, LY1.0, and LY1.5 treatment groups were statistically significantly greater than the placebo group (-0.89±0.12%, -1.03±0.11%, and -1.04±0.13% vs. 0.01±0.13%, respectively, all p<0.001, Figure 1). Dose-dependent reductions were also observed in fasting plasma glucose (ranging from -2.08±0.27 mmol/L in LY1.5 mg to -0.21±0.25 mmol/L in placebo group, p<0.001). Percent of patients achieving HbA_{1c} <7.0% in all groups at the 12 week endpoint were: placebo (21%), LY0.1 (47%), LY0.5 (73%), LY1.0 (75%), and LY1.5 (71%). A significant dose-dependent weight loss was demonstrated across LY2189265 doses (p=0.009), but none of the groups were different from the placebo group. Significant dose-dependent increases in homeostatic model assessment (HOMA2%B) were observed across LY groups (p=0.036) indicating improvement of beta cell function. Increases were significantly larger in each of the LY doses compared to placebo (p≤0.013) except the LY0.1 group (p=0.325). Overall, the most common adverse events were nausea 7.9% (n=13), diarrhea 6.1% (n=10), and nasopharyngitis 5.5% (n=9).

Conclusion: Treatment of patients with type 2 diabetes for 12 weeks with the long-acting GLP-1 analog LY2189265 as monotherapy resulted in dose-dependent reductions in HbA_{1c} and blood glucose with an acceptable safety profile of all doses.

Figure 1. HbA_{1c} change from baseline

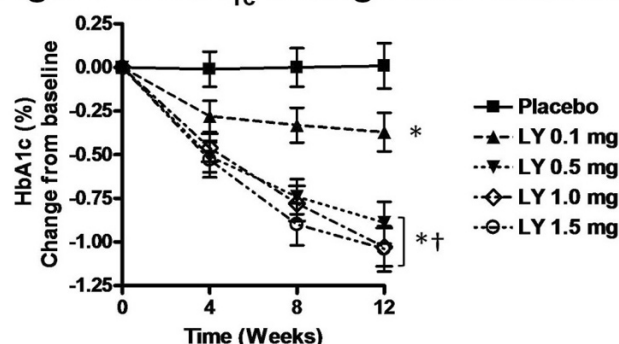


Figure 1. Least-squares mean (LSmean) change from baseline in percent HbA_{1c} by study visit (LSmean±SE). *p<0.05 versus baseline, †p<0.05 versus placebo.

Clinical Trial Registration Number: NCT00791479

789

Improved patient satisfaction with ITCA 650 vs. exenatide injections in subjects with metformin-treated type 2 diabetesT. Alessi¹, R.R. Henry², J. Rosenstock³, K. Luskey¹;¹Intarcia Therapeutics, Hayward, ²University of California at San Diego, La Jolla, ³Dallas Diabetes and Endocrine Center, Dallas, USA.

Background and aims: The relationship between exenatide treatment and patient satisfaction was examined in a study evaluating ITCA 650, a subcutaneous osmotic delivery system that provides for the continuous delivery of exenatide at specified doses for 3 months, and twice-daily exenatide injections.

Materials and methods: A 24-week phase 2 study was conducted in which patients were randomized to one of two doses of ITCA 650 (20 and 40 mcg/day) or exenatide injections (5 mcg BID x 4 weeks, 10 mcg BID x 8 weeks) administered for the first 12 weeks. In the second 12 weeks, subjects receiving exenatide injections were switched to ITCA 650 (40 and 60 mcg/day) and subjects receiving ITCA 650 were randomly assigned to either continue their previous dose or the dose was escalated to 60 or 80 mcg/day. The Diabetes Medications Satisfaction Tool (DM-SAT) was administered prior to initial treatment and at week 8 and week 20 of treatment. This questionnaire consists of 16 questions and evaluates overall satisfaction as well as satisfaction grouped into four subscales: lifestyle, well-being, glucose control and convenience.

Results: As indicated in the table, reductions in HbA_{1c} and weight were seen at week 12 in all groups and at week 8 the increase in overall satisfaction with treatment was greater among patients treated with either 20 or 40 mcg/d of ITCA 650 than with exenatide injections (25% and 40% vs.15%). A similar pattern was observed for the subscale scores where increased satisfaction with either dose of ITCA 650 was greater than the change with exenatide injections. At week 20, further improvements in HbA_{1c} and weight were seen with dose escalation and treatment satisfaction was maintained after the 2 - 3 fold dose escalation of ITCA 650. The most impressive results were observed among patients who started on exenatide injections and then switched to ITCA 650 where an average improvement of 20% in overall score was observed between week 8 (on exenatide injections for 8 weeks) and week 20 (on ITCA 650 for 8 weeks). GI side effects did not appear to impact the satisfaction score as an increase was also seen in subjects who reported nausea, similar to those that did not report nausea.

Conclusion: This study indicates that exenatide treatment was associated with an increase in patient satisfaction; however, the improvement was substantially greater with ITCA 650 compared to twice-daily injections. The ability to deliver exenatide in an injection-free manner that does not require any intervention by the patient ensures consistent compliance with prescribed treatment and results in increased satisfaction that may ultimately lead to improved persistence on long-term exenatide therapy. Evaluation of 6- to 12-month devices will be undertaken in phase 3 studies and may further enhance patient satisfaction.

in reducing FPG, HbA_{1c} and body weight (BW). The relationship between the PK and PD of ITCA 650 was examined.

Materials and methods: PK (plasma exenatide concentrations) and PD (FPG, HbA_{1c}, BW) data were collected from type 2 diabetics in a 28-day Phase 1b study (44 subjects) and a 24-week Phase 2 study (155 metformin-treated subjects) with a 24-week extension period. ITCA 650 doses ranged from 10-80 mcg/day in Phase 1b and 20-80 mcg/day in Phase 2. C_{ss} and AUC_{ss} were assessed from 24 hrs after device insertion through end of treatment. Exposure-response relationships were assessed with linear and tobit regression models, and graphically explored with LOESS (locally weighted scatterplot smoothing) techniques.

Results: There was a dose-proportional increase in exenatide exposure (AUC_{ss} and C_{ss}). Mean C_{ss} ranged from 38 ± 12 pg/mL at 10 mcg/day to 290 ± 117 pg/mL at 80 mcg/day. ITCA 650 provided continuous exposure for the duration of each device insertion (up to 12 weeks) and for the duration of treatment (up to 48 weeks). Exposures with ITCA 650 were consistent with exposures reported for SC infusion and BID exenatide injection. In contrast to BID exenatide injections, a pattern of peak and trough levels was not observed with ITCA 650. The effects of ITCA 650 were apparent within 24 hrs of device insertion. Plasma exenatide was detected in 32 of 44 Phase 1 subjects within 6-12 hrs, and steady state concentrations were achieved within 24 hrs in all dose groups. Reductions in FPG were observed within one day. FPG and 2-hr postprandial glucose were significantly decreased in the 20, 40 and 80 mcg/day dose groups within 1-5 days of initiating ITCA 650 and throughout the 28-day treatment period (p<0.05). The PK/PD relationship of exenatide exposure relative to changes in HbA_{1c} and BW was examined in Phase 2. All doses (20-80 mcg/day) were active suggesting an ED₅₀ of < 20 mcg/day. Higher exenatide concentrations tended to produce larger reductions in HbA_{1c} and BW; concentrations associated with all doses were on the relatively flat upper part of the response curve. These PD responses were maintained over 48 weeks of treatment. Baseline HbA_{1c} also contributed to the change in HbA_{1c}. In the combined population from all dose groups, subjects in the lowest quartile of starting HbA_{1c} values had a mean HbA_{1c} of 7.0% and a change in HbA_{1c} of -0.7% whereas subjects in the highest quartile had a HbA_{1c} of 9.2% and a mean change in HbA_{1c} of -1.9%. Consistent with its 2.4 hr half-life, exenatide was not measurable in any subject 24 hrs after ITCA 650 device removal. Samples collected at the next visit 7 days later confirmed that FPG levels had returned to baseline values.

Conclusion: ITCA 650 provides continuous exposure to exenatide for the duration of device insertion, dose-proportional exposures, rapid attainment of steady state plasma exenatide following device insertion and rapid clearance of exenatide after device removal. A relationship between exenatide exposure and glycemic and weight parameters was noted. In addition, the baseline HbA_{1c} played a role in the magnitude of the response to ITCA 650 treatment.

Clinical Trial Registration Number: NCT00943917

Changes in HbA_{1c}, Weight and DM-SAT Scores at Weeks 8-12

	Change in HbA _{1c} at Week 12	Change in Weight at Week 12	Baseline DM-SAT Score	Week 8 DM-SAT Score	Change in DM-SAT Score	Improvement in DM-SAT Score
ITCA 650 20 mcg/day	-1.0±0.9	-0.8±2.4	56.1±19.5	69.9±14.8	13.8±17.6	25%
ITCA 650 40 mcg/day	-1.0±0.7	-2.0±3.1	49±20.6	68.8±15.7	19.8±22.2	40%
Exenatide Injections	-0.8±0.9	-1.3±2.5	53±17.8	60.9±16.3	7.9±17	15%

Clinical Trial Registration Number: NCT00943917

790

Pharmacokinetic and pharmacodynamic assessments with ITCA 650, a continuous subcutaneous delivery of exenatide via DUROS[®] device, in type 2 diabetesJ. Dahms¹, Y. Chandrasekher¹, R. Fielding², R. Zhou³, R.R. Henry⁴, J. Rosenstock⁵, T. Alessi¹, K. Luskey¹;¹Intarcia Therapeutics, Hayward, ²Biologistic Services, Boulder, ³Medpace, Cincinnati, ⁴University of California at San Diego, La Jolla, ⁵Dallas Diabetes and Endocrine Center, Dallas, USA.

Background and aims: ITCA 650 is a subcutaneous osmotic delivery system that provides for the continuous delivery of exenatide. ITCA 650 is effective

PS 064 Liraglutide: clinical studies and observations

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The impact of disease stage, indicated by number of previous oral antidiabetic agents, on the clinical benefits of liraglutide in the treatment of type 2 diabetes

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Background and aims: The optimal point for GLP-1 receptor agonist therapy of type 2 diabetes is unclear. This analysis seeks to determine efficacy differences between early liraglutide addition (add-on to patients on ≤ 1 oral antidiabetic agent [OAD]) vs later liraglutide addition (add-on to ≥ 2 OADs) in patients with T2D.

Materials and methods: To evaluate the effect of placebo or liraglutide (1.2 mg or 1.8 mg), a pooled analysis of 26-week data from 7 randomised phase 3a and 3b clinical trials (n=4625) was carried out. Patients continued pre-trial OAD treatment throughout the trial; analyses were stratified according to pre-trial treatment status: diet/exercise or add-on to one OAD (Early), vs add-on to ≥ 2 OADs (Late). Beta-cell function was assessed by HOMA-B and proinsulin:insulin ratio.

Results: The mean duration of diagnosed diabetes was 6 years in the early group and 9 years in the late addition group. Change in HbA_{1c} from baseline was significantly greater in all early vs late patient treatment cohorts; for patients receiving liraglutide 1.8 mg, treatment difference (95% confidence interval [CI]): -0.36 [-0.52, -0.20]; $p < 0.0001$ (Table). A similar trend was observed in patients receiving liraglutide 1.2 mg and placebo. A significantly higher proportion of patients receiving liraglutide 1.8 mg early reached HbA_{1c} target $< 7\%$ than those treated late (Table). There was a significantly greater improvement in HOMA-B among patients treated early vs late with liraglutide 1.8 mg (Table). These differences persisted after correction for differences in baseline HbA_{1c}, weight and other parameters.

Conclusion: Liraglutide administration to patients who were treatment naive or previously receiving only 1 OAD produced greater glycaemic efficacy than in patients taking 2 or more OADs. This analysis suggests that use of liraglutide early in type 2 diabetes may provide greater clinical benefit and potential improvement in beta-cell function as compared with liraglutide treatment later in the disease process.

		LS Means		Difference Early vs Late (95% CI)	p value
		EARLY N=987	LATE N=495		
Change in HbA _{1c} from baseline (%)	Lira 1.8 mg	-1.55	-1.18	-0.36 (-0.52, -0.20)	$p < 0.0001$
	Lira 1.2 mg	-1.38	-0.82	-0.56 (-0.92, -0.19)	$p = 0.0027$
	Placebo	-0.46	0.09	-0.55 (-0.87, -0.22)	$p = 0.0010$
Change in HOMA-B from baseline (%)	Lira 1.8 mg	41.3	23.8	17.5 (5.7, 29.2)	$p = 0.0037$
	Lira 1.2 mg	36.3	26.4	9.9 (-12, 31.8)	$p = 0.3740$
	Placebo	1.4	-6.4	7.8 (-14.9, 30.5)	$p = 0.5018$
Change in proinsulin:insulin from baseline	Lira 1.8 mg	-0.11	-0.06	-0.05 (-0.1, 0.0)	$p = 0.0535$
	Lira 1.2 mg	-0.08	0.05	-0.03 (-0.14, 0.09)	$p = 0.6504$
	Placebo	0.02	0.01	0.01 (-0.07, 0.09)	$p = 0.8130$
Change in HOMA-IR from baseline	Lira 1.8 mg	-0.92	-0.94	0.02 (-0.81, 0.85)	$p = 0.9569$
	Lira 1.2 mg	-0.94	-1.04	0.10 (-1.55, 1.75)	$p = 0.9074$
	Placebo	1.15	-0.18	1.34 (-0.17, 2.84)	$p = 0.0813$
		EARLY	LATE	OR (95% CI)	p value
Proportion of subjects reaching HbA _{1c} $< 7\%$ (%)	Lira 1.8 mg	72	49	2.76 (1.75, 4.34)	$p < 0.0001$
	Lira 1.2 mg	61	41	2.28 (0.74, 7.03)	$p = 0.1526$
	Placebo	14	9	1.78 (0.69, 4.58)	$p = 0.2311$

Clinical Trial Registration Number: NCT00318422; NCT00318461; NCT00294723; NCT00333151; NCT00331851; NCT00518882; NCT00700817
Supported by: Novo Nordisk

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After one year of treatment, liraglutide more successfully reduces HbA_{1c} than sitagliptin across a broad range of baseline HbA_{1c} values, when both are combined with metformin

M. Davies¹, R. Pratley², E. Montanya Mias³, Y. Xu⁴, H. Hartvig⁵, G. Sesti⁶

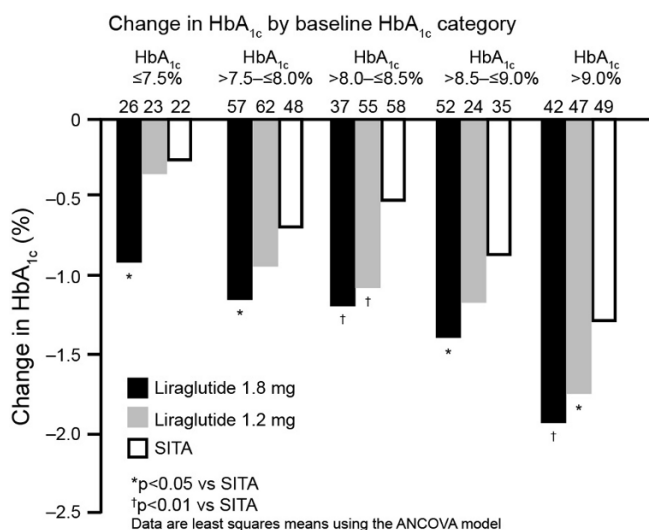
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Background and aims: Baseline HbA_{1c} is an important factor in therapy choice and also influences the magnitude of response to diabetes treatment. The aim of this study was to perform a post-hoc analysis to determine the impact of baseline HbA_{1c} on final HbA_{1c} following liraglutide or sitagliptin treatment in patients with type 2 diabetes.

Materials and methods: A 26-week randomised trial previously demonstrated that in comparison with sitagliptin 100 mg/day (SITA), liraglutide (LIRA) once daily (1.2 mg and 1.8 mg) produced greater reductions in HbA_{1c} from baseline. Furthermore, a post-hoc analysis showed that at the highest ($> 9.0\%$) and lowest ($< 8.0\%$) baseline HbA_{1c} values, both doses of LIRA were more effective at lowering HbA_{1c} than SITA. We report a similar post-hoc analysis following a 26-week extension of this randomised trial, in which patients continued treatment to which they were originally randomised. Five baseline HbA_{1c} ranges were used to categorise patients (see Figure).

Results: Over 52 weeks, LIRA (both 1.2 and 1.8 mg) and SITA improved glycaemic control across all baseline HbA_{1c} categories, with the greatest reductions observed at higher baseline HbA_{1c} levels (LS means from ANCOVA). Reductions in HbA_{1c} across baseline categories ranged from 0.3–1.3% for SITA, 0.4–1.8% for LIRA 1.2 mg, and 0.9–1.9% for LIRA 1.8 mg. End of treatment HbA_{1c} ranged from 7.1–8.2% for SITA, 6.9–7.8% for LIRA 1.2 mg, and 6.4–7.6% for LIRA 1.8 mg. LIRA 1.8 mg reduced HbA_{1c} significantly more vs. SITA in all baseline HbA_{1c} categories: ($p < 0.05$), while LIRA 1.2 mg reduced HbA_{1c} significantly vs. SITA in two baseline categories: > 8.0 – $\leq 8.5\%$ ($p = 0.005$) and $> 9.0\%$ ($p = 0.03$). There were no significant differences between LIRA 1.2 mg and LIRA 1.8 mg in any baseline HbA_{1c} category. Nausea was higher with LIRA (1.2 mg, 21.7%; 1.8 mg, 27.5%) than SITA (5.5%), but the incidence declined and rates were similar at 52 weeks. Minor hypoglycaemia (episodes/patient/year) was similar for LIRA 1.2 mg (0.143), 1.8 mg (0.154) and SITA (0.137).

Conclusion: Across a broad range of baseline HbA_{1c} values including at HbA_{1c} levels of $< 7.5\%$, LIRA more successfully reduced HbA_{1c} than SITA, over 1 year's treatment in combination with metformin.



Clinical Trial Registration Number: NCT00700817

Supported by: Novo Nordisk

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Liraglutide achieved greater weight reduction and improved glycaemic control compared with sitagliptin following one-year treatment when both are used in combination with metformin

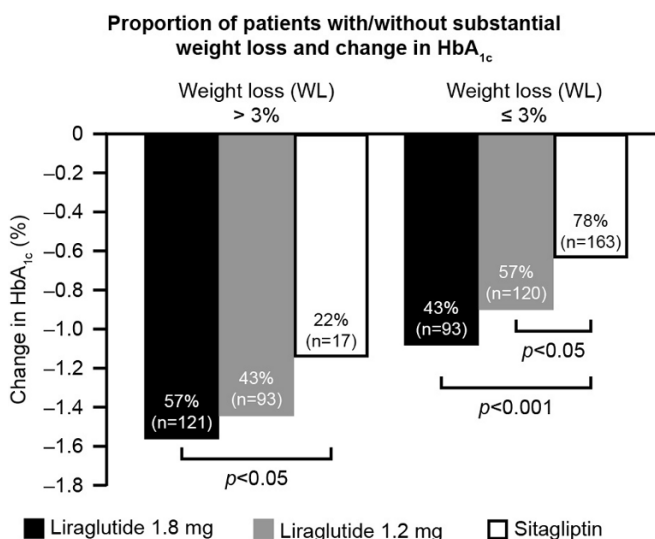
D.R. Matthews¹, G. Sesti², R. Pratley³, L. Grimmshave⁴, Y. Xu⁵, A. Garber⁶; ¹Oxford Centre for Diabetes, Endocrinology & Metabolism, The Churchill Hospital, Oxford, UK, ²University Magna Graecia of Catanzaro, Italy, ³University of Vermont College of Medicine, Burlington, USA, ⁴Novo Nordisk A/S, Copenhagen, Denmark, ⁵Novo Nordisk Inc, Princeton, USA, ⁶Baylor College of Medicine, Houston, USA.

Background and aims: Glucagon-like peptide-1 (GLP-1) receptor agonists are associated with weight loss in many patients while DPP-4 inhibitors have typically been shown to be weight-neutral. The results from a 26-week randomised head-to-head comparison study, liraglutide 1.2 mg and 1.8 mg once daily resulted in significant HbA_{1c} reduction and weight loss as compared to sitagliptin, both in combination with metformin. We report the results from the comparable post-hoc analysis following a 26-week extension of this randomised trial.

Materials and methods: During the trial extension, patients continued treatment to which they were originally randomised. 497/554 (90%) of completers from the initial 26-week study entered the 26-week extension phase. The effect of weight reduction category on HbA_{1c} was analysed using a post-hoc ANCOVA conducted on data from the full analysis set with last observation carried forward (LOCF).

Results: After 1 year's treatment, weight reductions in the total study population were significantly greater for LIRA 1.2 mg and 1.8 mg than SITA (-2.8 kg and -3.7 kg, vs -1.2 kg, respectively; $p=0.0001$). Patients who lost >3% BW had significantly greater reductions in HbA_{1c} compared with those who lost ≤3% BW in all treatment groups (Fig). Within each weight-loss group, treatment with LIRA 1.8 mg led to significantly greater reduction in HbA_{1c} vs SITA, and in the ≤3% weight-loss group LIRA 1.2 mg led to a significant reduction in HbA_{1c} vs SITA. The proportion of patients achieving the ADA target HbA_{1c} <7.0% was significantly greater with LIRA 1.8 mg in both groups vs. SITA and for 1.2 mg in the ≤3% group vs SITA.

Conclusion: One year of liraglutide treatment resulted in significantly higher proportion of patients achieving substantial weight loss than sitagliptin. HbA_{1c} reduction is significantly greater in patients with >3% weight loss with either treatment. HbA_{1c} reductions were significantly greater with LIRA 1.8 mg compared with SITA regardless of the level of weight loss achieved.



% represents proportion of patients in each treatment group in that weight loss category. Change in HbA_{1c} was significantly greater in WL ≤ 3% compared to WL > 3% for each treatment.

Clinical Trial Registration Number: NCT00700817

Supported by: Novo Nordisk

794

Liraglutide produces greater reductions in HbA_{1c} levels compared with sitagliptin or exenatide across five baseline HbA_{1c} categories

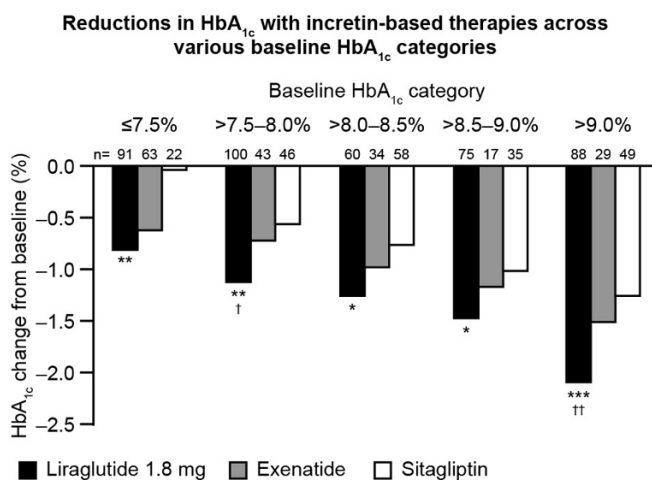
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Background and aims: Patients with higher baseline HbA_{1c} values demonstrate a greater reduction in absolute HbA_{1c} levels when treated with several type 2 diabetes therapies. When selecting therapeutic regimens for patients, physicians indicate that the availability of treatment comparisons can provide an invaluable resource to ensure that the most appropriate therapy is prescribed. We performed a meta-analytical assessment of patient level data of three incretin-based therapies in order to determine the effect of these therapies on HbA_{1c} levels based upon baseline HbA_{1c}.

Materials and methods: This meta-analysis comprised two phase 3 trials, one comparing liraglutide 1.8 mg once-daily with exenatide 10 µg twice-daily (both plus metformin [met] and/or sulphonylurea) and the other comparing liraglutide 1.8 mg (+met) with sitagliptin 100 mg (+met). Patients were allocated to five baseline HbA_{1c} categories: ≤7.5%, 7.5–8.0%, 8.0–8.5%, 8.5–9.0%, >9.0%. ANCOVA analysis (last observation carried forward, intention to treat population) was employed to estimate HbA_{1c} changes from baseline to 26 weeks, with respect to baseline HbA_{1c}. Country and interaction between treatment and HbA_{1c} category were included as fixed effects.

Results: HbA_{1c} improvements were observed for all treatments and across every HbA_{1c} category. Greater reductions were observed in higher baseline HbA_{1c} categories (figure). In the lowest baseline HbA_{1c} category (≤7.5%) reductions from baseline were 0.8% (liraglutide), 0.6% (exenatide) and 0.05% (sitagliptin), whereas greater reductions were observed in the highest baseline HbA_{1c} category (>9.0%), 2.1%, 1.5% and 1.2% respectively. Significantly greater reductions in HbA_{1c} were seen with liraglutide vs. sitagliptin across all HbA_{1c} categories. Liraglutide offered greater HbA_{1c} reductions vs. exenatide in all HbA_{1c} groups, with statistical significance being achieved in the 7.5–8.0% and >9.0% categories.

Conclusion: Reductions in HbA_{1c} were observed in all categories regardless of treatment, with greater reductions being observed in categories with higher baseline HbA_{1c} levels. Liraglutide offered significantly greater HbA_{1c} improvements compared with sitagliptin across every HbA_{1c} category and in all HbA_{1c} categories when compared with exenatide, two of which reached statistical significance.



* $p<0.01$, ** $p<0.001$, *** $p<0.0001$ vs sitagliptin

† $p<0.05$, †† $p<0.005$ vs exenatide

Clinical Trial Registration Number: NCT00518882; NCT00700817

Supported by: Novo Nordisk

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Greater glycaemic control is achieved with the once-daily human GLP-1 analogue liraglutide vs comparators across the continuum of estimated beta cell mass

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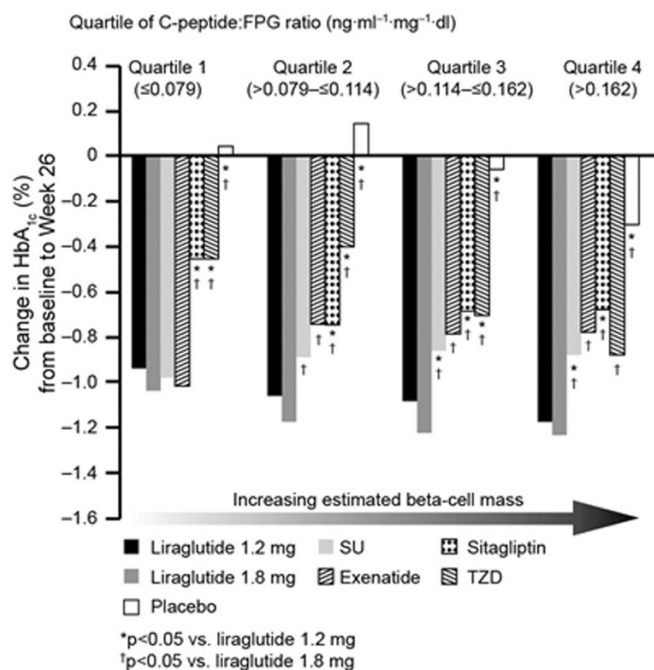
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Background and aims: Glycemic control is closely related to pancreatic β -cell mass in humans. A recent study showed that in patients with and without T2D, a C-peptide:fasting plasma glucose (FPG) ratio appears to be a better predictor of pancreatic β -cell area (as an estimate of β -cell mass) than measures such as the Homeostasis Model Assessment (HOMA) index. This analysis investigated the glycemic efficacy of the once-daily (OD) human glucagon-like peptide (GLP)-1 analog liraglutide (LIRA) vs other antidiabetic agents in patients with T2D as a function of estimated residual β -cell mass.

Materials and methods: A meta-analysis of patient level data was performed across 7 randomised phase 3 trials from the liraglutide development program, using data from 0–26 weeks. An ANCOVA model adjusted by quartile of residual β -cell mass (defined by C-peptide:FPG ratio) at baseline, was used to assess change in HbA_{1c} from baseline ($n=3941$) with LIRA 1.8 and 1.2 mg OD vs exenatide (EXEN), sitagliptin (SITA), sulfonylurea (SU), thiazolidinedione (TZD) or placebo in patients with T2D. A logistic regression model was used to assess percentage of patients achieving target $HbA_{1c} < 7\%$ by quartile of residual β -cell mass ($n=1577$) vs comparators. ANCOVA and the logistic regression model used trial, previous treatment and interaction between treatment and baseline C-peptide:FPG quartile as fixed effects and baseline HbA_{1c} as covariate.

Results: After 26 weeks' treatment with LIRA 1.8 mg, HbA_{1c} reduction from baseline and number of patients reaching target $HbA_{1c} < 7\%$ were significantly greater in all quartiles of estimated β -cell mass compared with sitagliptin, TZD or placebo (Fig). In patients with the greatest estimated β -cell mass at baseline (Quartile 4; Q4), both HbA_{1c} reduction and patients reaching target with liraglutide 1.8 mg (69.8%) were significantly greater at 26 weeks vs EXEN (49.7%), SU (49.8%), TZD (41.0%), SITA (21.3%) and placebo (21.7%). HbA_{1c} reduction and patients reaching target with LIRA 1.2 mg (61.3%) in Q4 were significantly greater than with SITA and placebo. In Q2 and Q3, the trend was towards greater HbA_{1c} reduction and a greater percentage of patients reaching target with both LIRA 1.2 and 1.8 mg vs comparators. In patients with the lowest estimated β -cell mass at baseline (Q1), HbA_{1c} reduction and patients reaching target were similar between LIRA, SU and EXEN. However, HbA_{1c} reduction and patients reaching target in Q1 were significantly greater with LIRA 1.8 mg (57.3%) vs SITA (20.6%), TZD (29.5%) and placebo (14.9%).

Conclusion: These data demonstrate that in patients with T2D, glycemic control with liraglutide is effective across the continuum of estimated β -cell mass. In particular, this finding implies enhanced glycemic benefits with liraglutide vs comparators, in patients at early and later stages of T2D disease progression.



Clinical Trial Registration Number: NCT00318422; NCT00318461; NCT00294723; NCT00333151; NCT00331851; NCT00518882; NCT00700817
Supported by: Novo Nordisk

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In patients with type 2 diabetes, overall treatment satisfaction improves following a switch from sitagliptin to liraglutide treatment in combination with metformin

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Background and aims: Patient-reported outcomes (PRO) from clinical trials provide insight into how treatments meet patients' needs and expectations. It has previously been shown that 1-year treatment with liraglutide (LIRA) 1.2 mg or 1.8 mg OD led to superior glycaemic control and weight loss vs sitagliptin (SITA) 100 mg OD, both added to metformin (MET) ≥ 1500 mg. During a 26-week extension period, patients treated with SITA randomly switched to LIRA 1.2 or 1.8 mg, resulting in further HbA_{1c} reductions and weight loss (HbA_{1c} : -0.24%, -0.45%; weight: -1.6 kg, -2.5 kg for SITA→LIRA 1.2 and 1.8 mg, respectively). Here we report PRO results from a pre-defined sub-population of this trial.

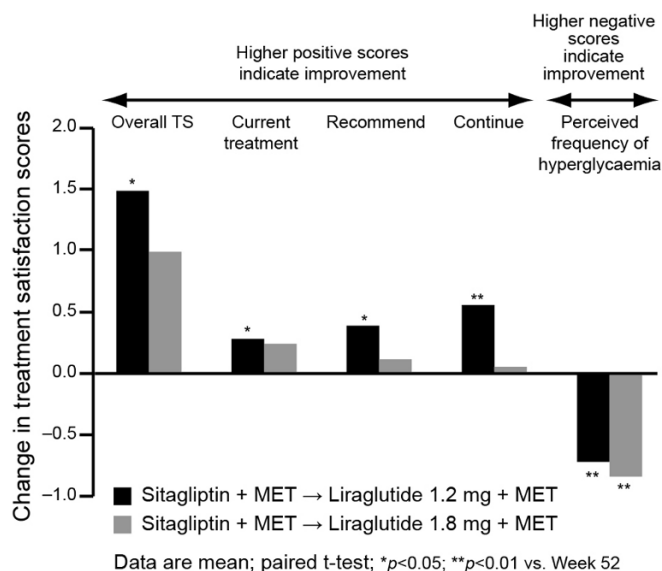
Materials and methods: Treatment satisfaction (TS) was evaluated using the Diabetes Treatment Satisfaction Questionnaire (DTSQ) at 52 and 78 weeks to assess the impact of switching from an oral therapy to an injectable one. Overall TS was calculated by adding satisfaction scores for 'current treatment', 'convenience', 'flexibility', 'understanding', 'recommend', and 'continue'. Higher scores indicated improved TS. For evaluation of perceived frequency of hyper- and hypoglycaemia, lower scores indicate improvement. Changes in TS scores were analysed by paired *t*-test.

Results: In total, 102 subjects were included in this PRO analysis (SITA→LIRA 1.2 mg, $n=54$; SITA→LIRA 1.8 mg, $n=48$). Overall TS improved in both groups of patients who switched from SITA to LIRA (SITA→LIRA 1.2 mg, 1.48 [$p=0.0170$]; SITA→LIRA 1.8 mg, 0.98 [$p=NS$]; Figure) and was driven largely by the categories 'recommend' (SITA→LIRA 1.2 mg, 0.39 [$p=0.0025$]; SITA→LIRA 1.8 mg, 0.12 [$p=NS$]; Figure) and 'continue' present treatment (SITA→LIRA 1.2 mg, 0.56 [$p=0.0099$]; SITA→LIRA 1.8 mg, 0.06 [$p=NS$]; Figure); no statistical difference was observed between LIRA 1.2 mg and 1.8 mg after the switch. Significant reductions in 'perceived frequency of hyperglycaemia' were observed in both groups (figure). 'Convenience', 'flexibility',

‘understanding’ and ‘perceived hypoglycaemia’ were unchanged after switching from oral to injectable treatment.

Conclusion: Switching from an oral therapy to an injectable did not negatively affect treatment satisfaction; on the contrary patients who switched to LIRA had an increase in overall TS (significant for 1.2 mg). The greater treatment satisfaction with liraglutide may be facilitated by weight loss and the improved treatment efficacy or perception thereof.

Figure: Change in mean TS scores from Week 52 to Week 78 for patients switching from sitagliptin to liraglutide



Parameter	Before Liraglutide (n=9)	After Liraglutide (n=9)	P value
PASI	3.9 (2.6-7.4)	3.0 (0.5-6.0)	0.031
DLQI	4.0 (0.0-6.8)	1.5 (0.0-4.5)	0.023
iNKT (%CD3)	0.19 (0.13-0.29)	0.40 (0.34-0.56)	0.022
Weight (kg)	145 (104-187)	140 (99-177)	0.250
HbA1c (%)	8.3 (6.0-8.6)	7.2 (5.6-7.9)	0.018
HOMA-IR	7.8 (3.7-15.3)	3.9 (1.8-9.5)	0.043

kg, kilogrammes. HOMA-IR, Homeostatic Model of Insulin Resistance. PASI, Psoriasis Area and Severity Index. DLQI, Dermatology Life Quality Index. iNKT, circulating Invariant Natural Killer T cell. %CD3, percentage of T lymphocytes.

Data are expressed as median (interquartile range). P values were calculated using the related-samples Wilcoxon Signed Rank Test.

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797

Liraglutide decreases psoriasis severity

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Background and aims: Gastric bypass surgery leads to an increase in endogenous glucagon-like peptide 1 (GLP-1) levels and can lead to resolution of psoriasis. Invariant natural killer T (iNKT) cells are a rare subset of innate T cells with multiple immunoregulatory properties that are implicated in the pathogenesis of inflammatory disorders. We noted improvement in psoriasis severity in a patient who commenced glucagon-like peptide-1 (GLP-1) analogue therapy to optimise glycaemic control. We have previously found that the number of circulating iNKT cells is low in people with either severe obesity and in people with psoriasis. We have shown that gastric bypass surgery results in an increase in circulating iNKT cell number. We hypothesized that GLP-1 analogue therapy decreases psoriasis severity and increases circulating iNKT cell number.

Materials and methods: Nine people with both diabetes and psoriasis self-administered injections of the GLP-1 analogue, liraglutide, for 8 weeks (0.6mg daily for 2 weeks, then 1.2mg daily). Psoriasis severity was assessed before and 8 weeks after commencing liraglutide therapy using the psoriasis area and severity index (PASI) and the dermatology life quality index (DLQI) questionnaire. The change in circulating iNKT cell number was quantified by flow cytometry. Approval to conduct this study was obtained from the local research ethics committee.

Results: Seven of the participants were male. The median age was 54 years and the median BMI was 46kg/m². Liraglutide therapy led to decreases in the indices of psoriasis severity (PASI and DLQI) and to an increase in circulating iNKT cell number (see table). Weight did not change significantly with liraglutide therapy although HbA1c and the homeostatic model of insulin resistance (HOMA-IR) decreased considerably.

Conclusion: This pilot prospective cohort study suggests that incretin therapy ameliorates psoriasis severity. This effect may be mediated by the improvement in insulin resistance or by the action of GLP-1 on iNKT cells.

PS 065 GLP-1 receptor agonists: clinical practice and safety

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Achieving glycaemic control and weight loss with incretin-based therapies: a comparison of liraglutide, exenatide, and sitagliptin

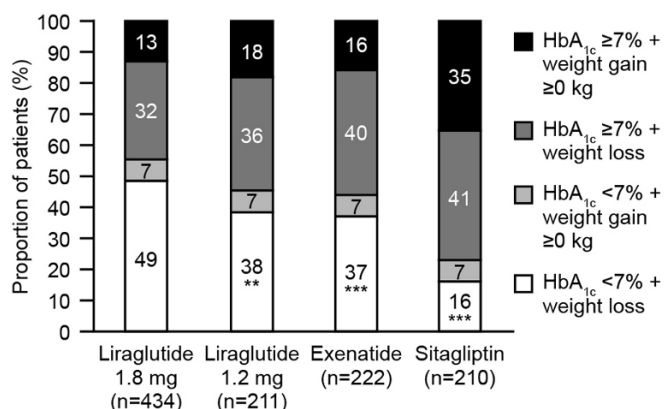
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Background and aims: Weight gain is a common undesirable outcome associated with several traditional antihyperglycaemic agents used for the treatment of type 2 diabetes. However, incretin-based therapies have been reported to provide enhanced glycaemic control with either weight neutrality (dipeptidyl peptidase-4 inhibitors) or weight loss (glucagon-like peptide-1 receptor agonists).

Materials and methods: We used patient-level data from two large randomised trials to compare the number of patients reaching the composite endpoint of HbA_{1c} levels <7% and weight loss (i.e. weight change <0 kg) after 26 weeks' treatment with liraglutide 1.8 mg or 1.2 mg once daily, exenatide 10 µg twice daily, and sitagliptin 100 mg once daily, all with metformin +/-sulphonylurea as background therapies. A logistic regression analysis with treatment and country as fixed effects and baseline HbA_{1c} and baseline body weight as covariates was performed on an intent-to-treat basis with missing data imputed (last observation carried forward).

Results: Mean baseline HbA_{1c} values were 8.1–8.5%. Reductions in HbA_{1c} from baseline were, in order of decreasing magnitude: liraglutide 1.8 mg (1.3%), liraglutide 1.2 mg (1.1%), exenatide and sitagliptin (both 0.9%). Mean baseline body weights were 93.0–93.8 kg. Reductions from baseline in body weight were, in order of decreasing magnitude: liraglutide 1.8 mg (3.0 kg), liraglutide 1.2 mg (2.7 kg), exenatide (2.3 kg) and sitagliptin (0.8 kg). More patients reached the composite endpoint (HbA_{1c} levels <7% and weight loss) with liraglutide 1.8 mg compared with all the other groups as indicated by the odds ratios and 95% CI: liraglutide 1.2 mg, 1.66 [1.14, 2.41], *p*<0.01; exenatide, 2.10 [1.41, 3.14], *p*<0.001; and sitagliptin 5.70 [3.63, 8.94], *p*<0.001 (Figure).

Conclusion: Patients are more likely to achieve glycaemic control with weight loss on liraglutide 1.8 mg than exenatide or sitagliptin.



p-values are from logistic regression analysis;
p*<0.01 and *p*<0.001 vs liraglutide 1.8 mg

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Treatment with exenatide BID and QW for 30 weeks was associated with favourable lipid subclass changes

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Background and aims: Cardiovascular (CV) disease risk is heavily influenced by metabolic abnormalities, especially dyslipidemia. Lipoprotein subclasses have become increasingly important in the evaluation of intermediate CV risk. Type 2 diabetes mellitus, which continues to increase in prevalence worldwide, is frequently accompanied by atherogenic dyslipidemia that increases risk for CV events. Treatment of type 2 diabetes with the GLP-1 receptor agonist exenatide improves HbA_{1c} as well as body weight, blood pressure, and lipids. This posthoc analysis investigated the effects of exenatide on lipoprotein subclasses.

Materials and methods: Of the 295 ITT patients with type 2 diabetes who participated in DURATION-1, a 30-week, controlled trial of exenatide BID (ExBID) and once-weekly exenatide (ExQW), 211 patients were assessed for lipid subclass changes using the Vertical Auto Profile ultracentrifuge method (n=106 ExQW; n=105 ExBID; 42% female, baseline HbA_{1c}: 8.2±1.0%; baseline weight: 100±19 kg; mean±SD). Baseline to end-of-study lipid changes were initially analyzed as unadjusted change scores using the SPSS General Linear Model, and then by adjusting for changes in HbA_{1c} and body weight.

Results: After 30 weeks of therapy, HbA_{1c} was reduced (LS mean [95%CI]) with ExQW by -1.6% (-1.8, -1.5) and with ExBID by -1.2% (-1.4, -1.1); weight was reduced by -3.9 kg (-4.9, -2.9) with ExQW and by -3.8 kg (-4.8, -2.8) with ExBID. Total cholesterol was not changed with either treatment. HDL and the subclass HDL-2 were significantly improved with ExQW (Table). Of note, HDL-2 was improved after adjustment for change in HbA_{1c} and weight. No change in HDL-3 or ApoA1 occurred with either treatment. LDL was not changed with either treatment; however, ExQW significantly reduced the smaller, denser, atherogenic subclass LDL-4 (even after adjustment for HbA_{1c} and weight change). Furthermore, ExQW significantly reduced ApoB and ApoB/ApoA1 ratio; these improvements remained after adjustment for HbA_{1c} and weight. Both treatments significantly improved triglycerides and VLDL (even after adjustment for HbA_{1c} and weight change). There were no changes in lipoprotein(a), IDL, or non-HDL. Adverse events (ITT population) were primarily gastrointestinal (nausea, vomiting, diarrhea), with a low rate of minor hypoglycaemia (6% incidence) and no major hypoglycaemia.

Conclusion: In this posthoc analysis, ExBID was associated with significant improvements in triglycerides, VLDL and ApoB/ApoA1. ExQW resulted in a more robust lipid improvement, with significant improvements in HDL, HDL-2, LDL-4, triglycerides, VLDL, and ApoB/ApoA1 after 30 weeks of treatment. All of these improvements, except HDL, were statistically significant even after adjustment for changes in weight and HbA_{1c}.

Δ Baseline to Week 30	ExQW	ExQW (adjusted)	ExBID	ExBID (adjusted)
ΔHDL (mg/dL)	+1.34 (0.07, 2.61)*	+1.25 (-0.01, 2.50)	+0.11 (-1.16, 1.39)	+0.21 (-1.05, 1.47)
ΔHDL-2	+0.52 (0.05, 0.99)*	+0.52 (0.06, 0.98)*	+0.21 (-0.27, 0.68)	+0.21 (-0.25, 0.67)
ΔHDL-3	+0.76 (-0.19, 1.72)	+0.70 (-0.27, 1.66)	-0.16 (-1.12, 0.80)	-0.09 (-1.06, 0.87)
ΔLDL (mg/dL)	-2.45 (-7.01, 2.10)	-2.81 (-7.41, 1.80)	+0.01 (-4.57, 4.59)	+0.37 (-4.27, 5.00)
ΔLDL-1	-0.25 (-1.29, 0.79)	-0.31 (-1.37, 0.74)	-0.30 (-1.35, 0.74)	-0.25 (-1.31, 0.81)
ΔLDL-2	+1.03 (-0.15, 2.22)	+0.91 (-0.29, 2.12)	+0.36 (-0.83, 1.55)	+0.48 (-0.73, 1.69)
ΔLDL-3	-0.44 (-2.74, 1.86)	-0.77 (-3.09, 1.55)	+1.58, (-0.73, 3.89)	+1.91 (-0.42, 4.25)
ΔLDL-4	-1.89 (-3.65, -0.14)*	-1.99 (-3.77, -0.21)*	-0.68 (-2.45, 1.08)	-0.59 (-2.37, 1.20)
ΔTriglycerides (mg/dL)	-35.2 (-53.7, -16.7)*	-31.9 (-50.1, -13.7)*	-26.7 (-45.3, -8.1)*	-30.0 (-48.3, -11.7)*
ΔVLDL (mg/dL)	-12.8 (-14.1, -11.6)*	-12.6 (-13.9, -11.4)*	-13.0 (-14.3, -11.8)*	-13.2 (-14.5, -12.0)*
ΔApoB/ApoA1 (%)	-3.1% (-5.0, -1.2)*	-3.1% (-5.0, -1.3)*	-1.0% (-2.9, 0.9)	-1.0% (-2.8, 0.9)

Mean (95%CI). **P*<0.05 vs. baseline. Adjusted = corrected for HbA_{1c} and weight change.

Clinical Trial Registration Number: NCT00308139

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Acute cardiovascular effects of exenatide in healthy male subjectsB. Mendis¹, E.J. Simpson², I.A. Macdonald², P.I. Mansell¹¹Department of Diabetes and Endocrinology, Nottingham University Hospitals NHS Trust, ²School of Biomedical Sciences, University of Nottingham, UK.

Background and aims: In large clinical trials with GLP-1 agonists and mimetics in patients with Type 2 diabetes mellitus (T2DM), a fairly consistent secondary outcome finding is of a moderate but clinically important fall in blood-pressure (ca.5/2mmHg), which precedes any weight loss. Exenatide is also being used to improve cardiac function in patients with left ventricular function and post cardiac surgery. The mechanism underlying the potentially beneficial cardiovascular effects of such drugs is clearly of interest but is not known. In rats, exendin-4, is weakly pressor with a marked increase in the vascular conductance in the hindquarters but vasoconstriction in the mesenteric and, to a lesser extent renal vascular beds. The aim of this study is to assess the acute cardiovascular effects of Exenatide in man.

Materials and methods: We studied 15 healthy non-diabetic, normotensive male subjects aged 18–45 years with BMI 20–27 kg/m² following responses to a single subcutaneous dose of exenatide 10mcg versus placebo. Measurements were made for 2 hours of heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), cardiac output (CO) and total peripheral resistance (TPR) by Finometer™. We also measured leg blood flow (LBF) by venous occlusion plethysmography and superior mesenteric artery (SMA) blood flow by Doppler ultrasonography. Arterialised venous blood samples were taken at 15 minute intervals.

Results: Data are given as mean ±SEM. With exenatide compared to placebo there was a greater increase from baseline in HR (change 10.4 ±1.1 beats per minute vs. 2.1 ±1.8 beats per minute, *P*<0.01), CO (change 1.7 ±0.2 l/min vs. 0.5 ± 0.2 l/min, *P*<0.01) and TPR (change -106 ±42.9 dyn.s/cm⁵ vs. 11 ±24.5, *P*< 0.05). There were no significant difference between exenatide and placebo in changes in SBP, DBP, LBF or SMA blood flow, with exenatide, blood glucose fell from 4.5 ±0.1 mmol/l to a nadir of 3.5 ±0.08 mmol/l at 45 minutes and finished at 4.2 ±0.08 mmol/l at 120 minutes.

Conclusion: A single dose of exenatide has significant cardiovascular effects in healthy subjects, in particular with an increase in CO and a decrease in TPR. These findings provide corroborative evidence to support the clinically observed modest hypotensive effect of exenatide in people with T2DM. Further studies are required to understand the mechanisms of the cardiovascular effects of exenatide in more detail.

Clinical Trial Registration Number: NCT01046721

801

Comparison of safety with continuous (exenatide once weekly) or intermittent (exenatide twice daily) GLP-1 receptor agonism in patients with type 2 diabetesT. Ridge¹, T. Moretto¹, L. MacConell², R. Pencek², J. Han², C. Schulteis², L. Porter²¹American Health Network, Indianapolis, ²Amylin Pharmaceuticals, Inc., San Diego, USA.

Background and aims: The glucagon-like peptide-1 (GLP-1) receptor agonist exenatide improves glycaemic control and promotes weight loss in patients with type 2 diabetes. Continuous or intermittent GLP-1 receptor agonism is achieved with exenatide once weekly (ExQW) or exenatide twice daily (ExBID), respectively. The objective of this post hoc pooled analysis was to examine safety and tolerability profiles of ExQW vs ExBID in 2 randomized, open-label studies (DURATION-1 and DURATION-5) of 30- or 24-weeks duration. In both studies, ExQW was demonstrated to be statistically superior to ExBID in reducing HbA1c over 30 or 24 weeks, respectively. In the intent-to-treat (ITT) population, least squares (LS) mean changes from baseline in HbA1c were -1.9% (ExQW) and -1.5% (ExBID) in DURATION-1 and -1.6% (ExQW) and -0.9% (ExBID) in DURATION-5, with LS mean treatment differences of 0.33% (DURATION-1) and 0.67% (DURATION-5). In both studies, subjects in both treatment groups lost weight, with LS mean improvements in weight ranging from -1.4 kg to -3.7 kg.

Materials and methods: The studies were conducted in 545 ITT patients (277 ExQW; 268 ExBID) with type 2 diabetes treated with diet/exercise or up to 2 oral antidiabetic medications (baseline [mean ±SD]: HbA1c 8.3 ±1.1%, fasting plasma glucose 9.2 ±2.5 mmol/L, weight 99 ±20 kg). At baseline, patients were drug-naïve (17%) or treated with one (45%) or a combination (38%) of oral

antidiabetes medications. Patients were randomized to receive ExQW (2 mg) or ExBID (5 µg [4 weeks] then 10 µg) over 24 or 30 weeks.

Results: The safety profiles of ExQW and ExBID were generally comparable. The incidence of serious adverse events (AEs; 4% in each group) and AEs leading to withdrawal (5% in each group) was low. The most common AEs were nausea (ExQW 21% vs ExBID 35%), diarrhea (ExQW 12% vs ExBID 9%), injection-site pruritus (ExQW 12% vs ExBID 1%), and vomiting (ExQW 8% vs ExBID 14%). These events were mostly mild/moderate in intensity. More ExBID (4%) than ExQW (1%) patients withdrew due to gastrointestinal AEs. Nausea occurred most frequently upon initiation of ExQW and at initiation and subsequent dose escalation of ExBID. Nausea incidence decreased with ongoing therapy in both groups; in the final 2 weeks of each study, new events of nausea occurred in <1% ExQW and <2% ExBID patients. Vomiting similarly decreased in incidence over time; in the final 2 weeks of each study, new events of vomiting occurred in <2% ExQW and <1% ExBID patients. Mild to moderate injection-site related AEs were more common with ExQW and generally occurred early (no new events after week 14). Two ExQW patients withdrew due to injection-site related AEs. No major hypoglycaemia occurred; minor hypoglycaemia occurred primarily in patients using a sulphonylurea (ExQW 13% vs ExBID 14%) and occurred at low incidence in patients not using a sulphonylurea (ExQW 0% vs ExBID <1%), with no difference between groups and no temporal association with initiation/dose escalation. There was no evidence of prolonged AE duration with ExQW vs ExBID.

Conclusion: Overall, continuous (ExQW) vs intermittent (ExBID) exenatide exposure did not impact the general safety profile of exenatide and both therapies were well tolerated. Less nausea and vomiting was observed with ExQW compared with ExBID. Injection-site related AEs were more frequent with ExQW, but rarely led to withdrawal (<1%).

Clinical Trial Registration Number: NCT00308139, NCT00877890

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Exenatide once weekly did not affect the QTc interval in patients with type 2 diabetesP. Sager¹, B. Darpo², J. Han³, B. Cirincione³, H. Linnebjerg⁴, M. Mitchell⁴, L. Porter³¹Sager Consulting Partners, San Francisco, USA, ²Dept. of Clinical Science and Education, Section of Cardiology, Stockholm, Sweden, ³Amylin Pharmaceuticals, Inc., San Diego, USA, ⁴Eli Lilly and Company, Indianapolis, USA.

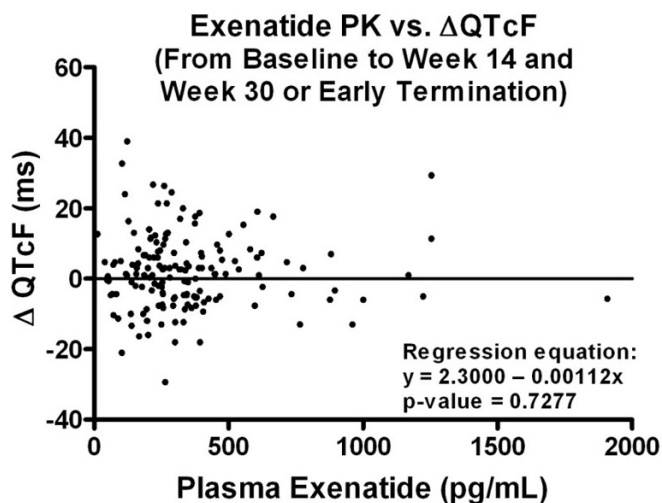
Background and aims: Exenatide once weekly (QW) is a GLP-1 receptor agonist under development for treatment of type 2 diabetes. The objective of this analysis was to evaluate cardiac repolarization in patients with type 2 diabetes treated with exenatide QW in a Phase 3 study.

Materials and methods: This analysis included 148 patients with type 2 diabetes (mean duration: 7 years) enrolled in the DURATION-1 Phase 3, randomized, open-label, comparator-controlled trial who were treated with exenatide QW (2 mg). Electrocardiograms (ECG) were recorded in triplicate and a concomitant blood sample was collected for exenatide concentration at baseline (prior to study treatment), week 14 (steady-state achieved), and week 30 or early termination. Blinded ECG overread by a cardiologist was performed at an independent core laboratory. The relationship between the Fridericia corrected QT (QTcF) and RR was examined to evaluate the adequacy of QTcF to minimize the confounding effect of heart rate. Consistent with Phase 3 studies, a pharmacological positive control for QT assessment was not included in the trial design; however, a sensitivity analysis of the established negative relationship between fasting plasma glucose (FPG) and change in QTcF (Δ QTcF) was performed to examine the ability to detect a small change in QTcF in this study. Mixed-model repeated measures method was applied to analyze the linear relationship.

Results: After 30 weeks of exenatide QW treatment, HbA1c was reduced by -1.9 ±0.1% (LS mean ±SEM). A small increase in heart rate (mean change [2-sided 90% CI]) was observed at 14 weeks (3.6 bpm [2.3, 4.8]; n=135) and 30 weeks (3.5 bpm [1.9, 5.0]; n=82). A shallow and statistically non-significant QTc/RR slope (*P*-value=0.08; slope: 0.0103) confirmed that the heart rate change was appropriately corrected by QTcF. A statistically significant negative relationship between FPG and Δ QTcF was detected (*P*-value=0.04; slope [95% CI]: -0.0526 [-0.1036, -0.0016]), indicating that the study was sufficiently sensitive to detect small changes in QTcF. The Δ QTcF (mean change [2-sided 90% CI]) was small and clinically insignificant at 14 weeks (1.7 ms [0.3, 3.1]) and 30 weeks (3.0 ms [0.9, 5.1]). No patient had a QTcF interval during treatment that exceeded 450 ms or a Δ QTcF >60 ms; 3 patients had a Δ QTcF >30 ms (30, 33, and 39 ms). The Δ QTcF was not accentuated in pa-

tients with the highest exenatide concentrations or renal insufficiency ($n=55$). Concentration-QTc analysis did not demonstrate a correlation between exenatide concentration and Δ QTcF (Figure).

Conclusion: In this study, exenatide once weekly treatment did not affect cardiac repolarization, measured by the QTcF interval, in patients with type 2 diabetes.



Clinical Trial Registration Number: NCT00308139

803

The risk of heart failure among patients receiving exenatide twice daily versus other glucose-lowering medications for diabetes: a matched retrospective analysis of the GE Healthcare EMR data

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Background and aims: Exenatide twice daily (ExBID), a glucagon-like peptide-1 (GLP-1) receptor agonist, has demonstrated improvements in cardiovascular risk factors in patients with diabetes. We hypothesized that the addition of ExBID to other glucose-lowering therapies may reduce the risk of developing heart failure, defined as diagnosis of ICD-9 code 428 or brain natriuretic peptide >100 pg/mL. This retrospective matched cohort study used data obtained from the national Medical Quality Improvement Consortium of ambulatory medical practices (>14,000 providers) that use Centricity Office from GE Healthcare IT as their electronic medical record.

Materials and methods: Patients with diabetes receiving a prescription for glucose-lowering therapy (ExBID, insulin [INS], and/or other [OTH, excluding ExBID and INS]) between 1 Jan 2005 and 30 Sept 2010 were identified ($n = 778,408$). Therapies may have been prescribed serially or concomitantly. Patients using ExBID were randomly matched 1:1 to patients not receiving ExBID based on gender, 10 year age band, follow-up time, and any use of thiazolidinediones. Odds ratios (OR) were calculated using conditional logistic regression models with and without adjustment for weighted Charlson Comorbidity Index (CCI), a disease severity measure.

Results: Without adjustment for CCI, the rate of heart failure (affected/total) among patients that received ExBID+INS+OTH was 4.15 vs 8.88 for INS+OTH (OR = 0.41; 95% CI = 0.33-0.50). The rate of heart failure among patients that received ExBID+OTH was 1.54 vs 2.34 in matched controls (OR = 0.66; 95% CI = 0.58-0.75). After adjustment for CCI, risk of heart failure for patients who received ExBID+INS+OTH was 57% lower vs INS+OTH (OR = 0.43; 95% CI = 0.40-0.47; $n = 48,184$). With adjustment for CCI, the risk of heart failure for patients who received ExBID+OTH was 38% lower vs OTH (OR = 0.62; 95% CI = 0.54-0.70; $n = 53,354$). Finally, in a model adjusting for CCI that included all patients that received ExBID vs all non-ExBID controls, the risk of heart failure was 51% lower (OR = 0.49; 95% CI = 0.46-0.52; $n = 101,538$).

Conclusion: In this analysis the addition of ExBID to glucose-lowering regimens for the treatment of diabetes was associated with reduced risk of developing heart failure.

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The Association of British Clinical Diabetologists (ABCD) nationwide exenatide and liraglutide audits

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Background and aims: To compare use and efficacy of exenatide and liraglutide in two large scale nationwide audits of real clinical practice.

Materials and methods: Exenatide/liraglutide audits respectively: 128/64 centres across UK submitted anonymised data on 6717/3010 patients during 2007-2009/2009-2010. Previous exenatide users were excluded from liraglutide analysis leaving 2303 patients.

Results: Baseline characteristics of patients are shown in Table 1. All data expressed as exenatide/liraglutide. At 6 months, mean (SE) HbA1c reduction were 0.75(0.04) v 0.93(0.07)% (difference, $p=0.040$) among 3166 patients. Weight reduction were 6.5(0.1) v 3.7(0.2) kg (difference, $p<0.001$) among 2790 patients. All HbA1c and weight changes from baseline were significant ($p<0.001$). Exenatide/liraglutide data for cholesterol reduction were 0.16(0.03)/0.14(0.05) mmol/L, triglycerides reduction were 0.14(0.06)/0.26(0.10) mmol/L and systolic blood pressure reduction were 3.6(0.6)/4.6(0.9) mmHg. These were significant from baseline (at least $p<0.05$). There was no change in diastolic blood pressure in the exenatide audit but with liraglutide this fell by 1.2(0.5) mmHg ($p=0.023$). Baseline treatment use(discontinuation) was sulphonylurea 49.5/42.8(6.5/5.3)%, thiazolidinedione 27.1/20.5(13.4/7.5)%, DPP4 inhibitor 2.2/10.9(1.4/9.3)%, insulin 33.9/39(8.1/2.6)%.

Conclusion: These very large audits reveal the effectiveness of these agents in much heavier and more poorly controlled patients than those studied in clinical trials. Patients achieved greater HbA1c reduction but lesser weight reduction in the liraglutide audit as compared with the exenatide audit. However, there were lesser insulin and TZD discontinuation but greater DPP4 inhibitor discontinuation in the liraglutide audit. Contributors might have learnt from the previous use of exenatide to avoid over-reduction of diabetes treatment when initiating liraglutide

	Exenatide	Liraglutide	p-value
n	6717	2303	
Male (%)	54.9	54.1	0.491
Caucasian (%)	84.4	90.4	<0.001
Age (yrs)	54.9 (10.6)	55.4 (11.2)	0.033
Diabetes duration (yrs)	8 (5-13)	9 (5-13)	0.424
HbA1c (%)	9.47 (1.69)	9.32 (1.72)	0.001
Weight (kg)	113.8 (23.4)	111.1 (23.0)	<0.001
BMI (kg/m ²)	39.8 (8.0)	39.1 (7.5)	<0.001
Single oral therapy (%)	21.6	12.0	<0.001
Dual oral therapy (%)	27.6	28.1	0.709
≥3 oral therapy (%)	6.5	17.9	<0.001
On insulin (%)	33.9	39.8	<0.001

Results quoted as mean (SD) and median diabetes duration (inter-quartile range)

Supported by: Eli Lilly Ltd, Novo Nordisk Ltd

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Factors associated with HbA_{1c} and weight changes at 6 months in the Association of British Clinical Diabetologists (ABCD) nationwide exenatide and liraglutide audit

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Background and aims: Treatment with GLP-1 agonists in type 2 diabetes has the advantage of weight loss but they are not effective in every patient. Factors that help predict response to treatment is needed. ABCD conducted two nationwide audits on exenatide and liraglutide based on real clinical practice.

Materials and methods: Patients from both audits were pooled together for analyses. Univariate followed by multivariate analyses were performed to assess for factors that were associated with HbA1c and weight change after GLP-1 agonist treatment. Latest HbA1c and weight changes by 6 months were used as continuous response variables and were assessed against other continuous variables of baseline HbA1c, weight, weight or HbA1c change, patient age, diabetes duration, total insulin dose (logarithm-transformed) and insulin dose reduction. Categorical variables assessed were gender, ethnicity (Caucasian/South Asian/Afro-Caribbean), oral hypoglycaemic agent change (stopped or reduced/unchanged/started or increased) and insulin use (yes/no). To avoid limiting the multivariate analyses to only insulin patients, two models were assessed each for HbA1c and weight change, the first with all significant univariate variables and with the variable insulin use, the second with total insulin dose and insulin dose reduction.

Results: 9020 patients with 5407 and 5245 follow-up HbA1c and weight results were analysed. Univariate analyses showed HbA1c reduction being correlated with higher baseline HbA1c and inversely with baseline weight, weight reduction, diabetes duration, TZD reduction, insulin use, higher insulin dose reduction (all $p < 0.001$) and higher daily insulin dose ($p = 0.012$). Univariate analyses for weight reduction showed correlation with higher baseline weight, age and diabetes duration, TZD reduction, insulin use and higher insulin dose reduction, and inversely with baseline HbA1c, HbA1c reduction and South Asian or Afro-Caribbean ethnicity (all $p < 0.001$). Table 1 shows the results of stepwise regressions analyses. The HbA1c change model had 3982 patients with values of baseline HbA1c and weight, weight change, diabetes duration, TZD reduction and insulin use. The weight change model had 3089 patients with values of HbA1c change, baseline weight and HbA1c, ethnicity, age, diabetes duration, TZD reduction and insulin use. The models accounted for 22.0% and 9.5% of the variance of HbA1c change and weight change respectively.

Conclusion: Besides intuitive factors that affect HbA1c and weight outcomes, insulin-treated patients were found to have less HbA1c reduction but more weight reduction after treatment with GLP-1 agonists. Higher total daily insulin dose and longer diabetes duration were also associated with poorer HbA1c reduction. Table 1: Stepwise regression analyses of factors influencing HbA1c and Weight changes among patients treated with exenatide and liraglutide.

Factor	HbA1c reduction, stepwise regression among 3982 patients		Weight reduction, stepwise regression in 3089 patients	
	Adjusted T-value	Adjusted p-value	Adjusted T-value	Adjusted p-value
Baseline HbA1c	30.44	<0.001	-5.94	<0.001
Baseline Weight	-3.79	<0.001	13.29	<0.001
HbA1c change	-	-	-	NS
Weight change	-	NS	-	-
Age	-	-	2.06	0.040
Diabetes duration	-4.16	<0.001	3.25	0.001
Ethnicity	-	-	-	NS
TZD reduction	-7.96	<0.001	7.02	<0.001
Insulin use	-10.02	<0.001	7.06	<0.001
	Stepwise regression among 1134 patients		Stepwise regression among 1002 patients	
Total insulin dose (log)	-3.6	<0.001	-	NS
Insulin dose reduction	-3.5	<0.001	9.21	<0.001

Supported by: Eli Lilly Ltd and Novo Nordisk Ltd

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Life-years, QALYs, costs and numbers needed to treat associated with exenatide once weekly versus insulin and pioglitazone treatment for type 2 diabetes: an Archimedes model simulation

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Background and aims: Exenatide once weekly (ExQW) is a glucagon-like peptide-1 (GLP-1) receptor agonist that improves glycaemia in patients with type 2 diabetes (T2DM) while potentially eliciting weight loss and improvement in cardiovascular risk factors (blood pressure and plasma lipids). In

published trials, ExQW resulted in superior reduction in HbA1c compared to maximum daily doses of sitagliptin and pioglitazone (PIO) on metformin (MET) background, and to titrated insulin glargine. We used the Archimedes Model, a validated, clinically detailed model of physiology, disease, and healthcare delivery, to explore potential long-term benefits of ExQW and to evaluate savings from ExQW that might be achieved through reduced healthcare expenditures.

Materials and methods: We simulated 20 y of treatment (reported annually) in a virtual population ($n = 24,878$) based on individuals with T2DM drawn from the National Health and Nutrition Examination Survey who were on MET±sulphonylureas and who had not yet advanced to insulin (mean age 57 y, BMI 33 kg/m², weight 94 kg, duration of T2DM 9 y, baseline HbA1c 8%). The potential effects of 3 different treatment regimens were modeled at simulation start: 1) advancement to insulin at HbA1c ≥8% (treat to target HbA1c <7%), 2) addition of PIO, and 3) addition of ExQW. ExQW's effect on HbA1c, weight, blood pressure, and lipids was derived from four Phase 3 ExQW clinical trials. Direct medical costs (inpatient, outpatient, ambulatory, treatments) inflated to 2010 USD were derived from the Medicare Current Beneficiary Survey, as well as Medicare Part D data, www.drugstore.com, and published literature. Since ExQW is investigational, antidiabetic therapy costs for ExQW, insulin, and PIO were excluded from the analysis.

Results: At 20 y, undiscounted life years for ExQW, insulin, and PIO were 16.06, 15.87, and 15.81, respectively; quality-adjusted life years (QALYs) were 13.72, 13.52, and 13.46, respectively. Similar gains for ExQW over insulin and PIO were seen at 5 y and 10 y as well. ExQW demonstrated per person-year savings of \$465 and \$327 vs insulin and PIO, respectively, at 5 y, \$528 and \$415 at 10 y, and \$556 and \$539 at 20 y. The numbers of patients needed to treat to prevent one major adverse cardiovascular event (composed of myocardial infarction, stroke, and cardiovascular death) were 94, 53, and 43 for ExQW therapy vs insulin at years 5, 10, and 20, respectively, and 147, 104, and 90 for ExQW vs PIO.

Conclusion: These simulations showed increased life-years and QALYs for ExQW vs insulin and PIO, as well substantial healthcare cost savings. Use of ExQW reduced direct medical costs by \$300 to \$560 vs insulin and PIO per person year throughout the 20 y simulation period. These explorations through simulation modeling provide early indication of the potential of ExQW and underscore the need for confirmation through real-world clinical trials.

PS 066 Incretins and pathophysiology

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DPP-IV inhibitor vildagliptin increases the pancreatic beta cell mass in non-diabetic mice by directly regulating cell kinetics

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Background and aims: We previously reported that vildagliptin (VILDA) preserved the pancreatic beta cell function and cell mass in diabetic KK-A^y mice probably through its action on the cell kinetics regulation and the anti-oxidative/ER stress mechanism. In this study, to further clarify the proliferative effect of VILDA on beta cells by eliminating the influence of dysmetabolism, we assessed the mode of action of VILDA on beta cell function and cell mass in non-diabetic C57BL/6J mice.

Material and methods: Eight week-old male KK-A^y/TaJcl (KK-A^y) mice and C57BL/6Jcl (B6) mice were divided into VILDA-treated and -untreated (control) groups (n=5). Body weight (BW), food intake, fasted blood glucose (FBG), insulin (FIRI), TG, NEFA and active GLP-1 were measured every 2 weeks. Intraperitoneal insulin tolerance test (ipITT: KK-A^y 2IU/kg BW, B6 0.75IU/kg BW), oral glucose tolerance test (OGTT: 1g/kg BW) and glucose stimulated insulin secretion (GSIS) from isolated pancreatic islet was performed at 12 weeks. Gene expressions specific for the core area of pancreatic islet were analyzed by Laser Capture Microdissection method and real time RT-PCR at 12 weeks. The beta cell mass, cell proliferation and apoptosis were assessed by histological analysis including PCNA, 4HNE, CHOP and TUNEL immunostaining of the islet tissue.

Results: BW, food intake, FBG, FIRI, NEFA and active-GLP-1 were not different between the control and VILDA-treated groups in both KK-A^y and B6 mice. The increased plasma TG level and islet TG content were ameliorated by VILDA treatment in KK-A^y. IpITT demonstrated a significant augmentation of insulin sensitivity by VILDA in both strain of mice. VILDA ameliorated glucose tolerance and induced significantly higher plasma insulin at nearly all of observed points on OGTT in KK-A^y, but these observation were observed at only 15min in B6 mice. GSIS with 16.7mM glucose was more significantly facilitated in VILDA groups than in the controls (KK-A^y: 1.5±0.1 vs. 1.1±0.1 ng/ml/islet, p=0.045, B6: 2.2±0.1 vs. 1.7±0.1 ng/ml/islet, p=0.042). The pancreatic beta cell mass and islet insulin content were greater in VILDA-treated mice than in the control mice (beta cell mass: KK-A^y 5.0±0.4 vs. 2.9±0.4 mg, p=0.016, B6 1.2±0.1 vs. 0.8±0.2 mg, p=0.047). The mRNA levels of Hlx-9, PDX-1, and ERK1 associated with cell differentiation/proliferation were significantly higher in both VILDA treated mice than in the control mice. VILDA significantly down-regulated XBP-1 related with ER stress, Caspase3 and CAD gene expression related with cell apoptosis, and up-regulated GSH-Px and SOD2 gene expression related with anti-oxidative stress in KK-A^y, but not in B6 mice. Morphometric results for PCNA, 4HNE, CHOP and TUNEL observed corresponded with the data obtained in gene expression analysis.

Conclusion: The present results suggest that vildagliptin increases the pancreatic beta-cell mass in non-diabetic mice through directly accelerating cell differentiation/proliferation.

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Effect of DPP-4 inhibitor vildagliptin on pancreatic beta cell failure in beta cell-specific C/EBPβ transgenic mice

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Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibitor lowers blood glucose levels by increasing insulin secretion from β cells. Although the effect of DPP-4 inhibitor on glycemic control is quite clear, it remains to be determined whether this reagent improves the pathogenic features of type 2 diabetes, such as endoplasmic reticulum (ER) stress and the reduction of β cell mass. We have recently reported that accumulation of the transcription factor C/EBPβ in β cells causes ER stress and a reduction in β cell mass lead-

ing to β cell failure. The present study aimed to examine whether the DPP-4 inhibitor vildagliptin reduces ER stress and preserves pancreatic β cell mass in β cell-specific C/EBPβ transgenic mice.

Materials and methods: By inserting the cDNA of C/EBPβ downstream of rat insulin promoter 2, we obtained two lines of transgenic mice, namely TG1 and TG2, which are different from each other in terms of the expression levels.

Results: β cell-specific C/EBPβ transgenic mice (TG1), in which the expression of C/EBPβ was 3-fold higher than that in wild-type mice (WT), showed a non-fasting glucose level of approximately 200 mg/dl, with increased ER stress and reduced β cell mass. TG1 and WT were orally treated with or without vildagliptin (vilda) dissolved in water (0.6 mg/ml) from 4 weeks of age. Treatment with vilda in TG1 markedly ameliorated hyperglycemia and increased serum insulin levels (WT with vilda: 136 ± 4.0, WT without vilda: 132 ± 4.8, TG1 with vilda: 154 ± 11.1, and TG1 without vilda: 193 ± 7.2; p < 0.01). TG1 treated with vilda also showed a significant improvement in glucose tolerance concomitant with the increased insulin secretion compared to TG1 without treatment. Immunostaining analyses of insulin and glucagon of the pancreatic islets after 8 weeks treatment showed that TG1 treated with vilda exhibited a 2-fold increase in β cell mass compared to TG1 without treatment, although they still did not reach the WT level. We next established C/EBPβ-overexpressing β cell lines and, using the GLP-1 receptor agonist exenatide, examined the effect of activation of the GLP-1 pathway on C/EBPβ-mediated ER stress and the reduced β-cell mass. C/EBPβ overexpression induced ER stress, as detected by the phosphorylation of PERK, eif2α, and c-jun, and activated components of the apoptotic pathway, including CHOP and cleaved caspase-3. Treatment with exenatide (10 nM) markedly reduced ER stress and activation of the apoptotic pathway induced by C/EBPβ overexpression.

Conclusion: The DPP-4 inhibitor vildagliptin reduced the β cell failure in β cell-specific C/EBPβ transgenic mice by increasing β cell mass. This effect might be mediated through the reduction of ER stress and apoptosis in β cells.

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A metabolomic study of GLP-1 analogue and glucagon receptor (GCGR) antagonist in DIO mice

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Background: Agonizing the GLP-1 receptor and antagonizing the glucagon receptor are two therapeutic approaches for achieving improved glycemic control. In preclinical animal models both GLP-1R agonists and GCGR antagonists exhibit robust efficacy in lowering blood glucose levels. Although the two hormones control distinct pathways in regulating glucose production and disposal, recent studies have demonstrated that the two approaches are interdependent in achieving maximal therapeutic efficacy. These findings, however, do not imply that GLP-1 agonists and GCGR antagonists have identical effects on all aspects of glucose homeostasis. The two agents may have distinct effects on energy and glucose metabolism.

Aims: To investigate the pharmacologic contributions of directly agonizing the GLP-1 receptor or antagonizing GCGR on energy state and glucose homeostasis in diet-induced obese (DIO) mice. To gain molecular insight on the mechanisms of action by conducting metabolomic study using stable isotope-based dynamic metabolic profiling (SiDMap) to analyze key metabolic pathways.

Material and methods: GCGR Ab is a human monoclonal IgG that antagonizes GCGR. In cells expressing recombinant mouse GCGR, GCGR Ab inhibited glucagon-induced cAMP elevation (IC₅₀ ~1.8 nM). The GLP-1 analog is a GLP-1 PEGylated with a C-terminal cysteine-alanine extension to give GLP-1 compound 23 (EC₅₀ ~0.13 nM). DIO mice were injected i.p. with either PBS (vehicle), GLP-1-(23) 10 µg/mouse, or GCGR Ab 1 mg/kg. After the baseline blood glucose levels were measured mice were injected i.p. with uniformly labeled ¹³C glucose tracer ([U-¹³C]₆]-D-glucose 2 g/kg. At 90 min after tracer injection, blood was collected, and liver, skeletal muscle (gastrocnemius/soleus), kidney, and white adipose tissues (epididymal) were removed. Metabolism of [U-¹³C]₆-D-glucose produces several stable, non-radiating isotope-labeled intermediary metabolite species. These isotopomers were readily separated and measured by using gas chromatography/mass spectrometry techniques.

Results: Although the two agents induced rapid clearance of administered tracer glucose, the metabolic fate of the labeled glucose showed both overlap-

ping and distinct paths in mice treated with the two different anti-diabetic agents. The rapid disposal of the glucose tracer by both agents was mediated by pathways including increased glycolysis and TCA cycle anaplerosis, resulting in a surge in plasma lactate and glutamate levels. Both agents also increased glucose oxidation in the liver and kidney, producing ATP and carbon dioxide. GLP-1-(23) treatment uniquely exhibited increased pentose cycle flux in the liver and kidneys. This biochemical reaction yielded additional NADPH, which is required by de novo fatty acid synthesis. This is consistent with the report that GLP-1 stimulates glucose-derived de novo fatty acid synthesis during insulin release.

Conclusion: Our data indicate that the two agents exhibit differential effects on energy metabolism and certain aspects of glucose homeostasis in a DIO rodent model. These data contribute to an understanding of the benefits and limitations of these two therapeutic agents.

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Protective effects of the GLP-1 receptor agonist lixisenatide on ischaemia-reperfusion-induced myocardial infarction in an isolated rat heart model

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Background and aims: Endogenous glucagon-like peptide-1 (GLP-1) is an incretin peptide secreted from intestinal L-cells in response to food intake. It stimulates insulin release from the pancreas when blood glucose levels are elevated. In addition, there is some evidence that GLP-1 may have beneficial cardiovascular effects. We assessed the cardioprotective effect of lixisenatide, a novel synthetic GLP-1 receptor agonist, native GLP-1 (GLP-17-36 amide) and liraglutide in isolated Langendorff-perfused rat hearts during regional ischemia and reperfusion.

Materials and methods: We performed transient occlusion of the left anterior descending coronary artery for 45 minutes followed by a reperfusion period of 120 minutes to permit simultaneous recording of left ventricular pressure, contractility, heart rate and coronary flow. Infarct size was determined by planimetry.

Results: Administration of lixisenatide at 0.3 nM starting 10 minutes prior to and during reperfusion significantly reduced myocardial infarct-size by 36% ($p=0.0028$ vs vehicle control). Native GLP-1 and liraglutide (both also at 0.3 nM) reduced myocardial infarct-size by 32% ($p=0.0071$ vs vehicle control) and 29% ($p=0.0159$ vs vehicle control), respectively. The observed cardioprotective effect was not associated with a significant change in cardiac hemodynamics, particularly coronary flow. However, all GLP-1 receptor agonists stimulated myocardial glucose uptake during recovery from regional ischemia, suggesting a direct cardiac effect.

Conclusion: The novel GLP-1 receptor agonist lixisenatide offers protection against myocardial ischemia-reperfusion infarction in isolated rat hearts. This finding may represent a novel therapeutic benefit and supports the rationale to study lixisenatide in a cardiovascular outcome trial.

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Effect of linagliptin on infarction size and cardiac function in rats after myocardial ischaemia reperfusion

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Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibition has been reported to have beneficial effects on myocardial ischaemia. Mechanisms behind these effects could include the involvement of stromal cell-derived factor (SDF)-1 and/or incretin receptor-dependent pathways that improve tissue repair. In addition, as some antihyperglycaemic agents have been associated with adverse cardiovascular (CV) outcomes, it is critical to ensure that agents in development, such as the DPP-4 inhibitor linagliptin, do not carry an increased CV risk. The objective of this study was to evaluate the cardiac effects of linagliptin in a rat ischaemia-reperfusion injury (I/R) model.

Materials and methods: Rats were divided into three groups: sham, I/R and I/R plus linagliptin ($n=16-18$ per group). Linagliptin was given once daily (3 mg/kg) starting 30 days before I/R. I/R was induced by ligation of the left

anterior descending coronary artery for 30 min. Echocardiography was performed after 58 days and cardiac catheterization after 60 days.

Results: Linagliptin significantly reduced the proportion of infarcted tissue relative to the total area at risk (-21% ; $p < 0.001$) as well as the absolute infarction size (-18% ; $p < 0.05$). In addition, glucagon-like peptide-1 (GLP-1) levels were increased 18-fold ($p < 0.0001$) and DPP-4 activity was reduced by 78% ($p < 0.0001$). Left ventricular left end diastolic and systolic pressure as well as all echocardiography parameters were similar between groups, with a significant improvement of isovolumetric contractility indices (dp/dt_{min}) from -4771 ± 79 mmHg/s to -4957 ± 73 mmHg/s.

Conclusion: These data further support a cardioprotective function of linagliptin in the setting of acute myocardial infarction.

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GLP-1 receptor expression in the human thyroid gland

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Background and aims: Glucagon like peptide-1 (GLP-1) mimetic therapy induces C-cell hyperplasia and medullary thyroid tumors in rodents. We sought to establish if C-cells in human medullary thyroid carcinoma (MTC), C-cell hyperplasia (CCH), papillary thyroid carcinoma (PTC) or normal human thyroid express the GLP-1 receptor (GLP-1R).

Materials and methods: Expression of GLP-1R as well as the C-cell marker calcitonin was determined by immunofluorescent labeling of thyroid tissue samples including medullary thyroid carcinoma, C-cell hyperplasia, papillary thyroid carcinoma as well as normal thyroid sections. GLP-1R antibody validation was carried out by transfecting cell lines that do not express the GLP-1R with human GLP-1R, specificity being documented by Western Blotting and immunofluorescence.

Results: Normal thyroid. GLP-1R expression was detected in 35% of C-cells in 15 normal control thyroid lobes. A total of 1,979 C-cells (range 12-433 per section) were identified by calcitonin labeling in these sections. Medullary thyroid carcinoma. Colocalisation of GLP-1R and calcitonin was observed in a varying proportion of C-cells in 11 of the 12 MTC examined. In 6 cases, more than 70% of C-cells were immunoreactive for GLP-1R. C-cell hyperplasia. Thyroids from 4 individuals with reactive (to inflammatory) CCH showed clear labeling for GLP-1R in all cells positive for calcitonin. Also, GLP-1 R expression was detected in all 5 individuals with nodular/neoplastic CCH due to MEN-2A or FMTC germline mutations. In addition, GLP-1R immunoreactivity was found in the medullary thyroid microcarcinomas present in these cases. Papillary thyroid carcinoma. Unexpectedly, we also detected GLP-1R expression in thyroid follicular cells in 3 of 17 of the PTC examined.

Conclusion: In humans, the GLP-1R is expressed in ~30% of C-cells in normal thyroid and a higher proportion of C-cells in C-cell hyperplasia and medullary thyroid carcinomas. Of concern, GLP-1R expression is also present in ~20% of papillary thyroid carcinomas, the latter being present in ~8% of the adult population. While it is unknown if GLP-1 induces proliferation of follicular or C-cells expressing the GLP-1 receptor, it will be important to follow individuals treated with GLP1 based therapy to exclude an increase in the incidence of medullary or papillary thyroid carcinomas.

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The GLP-1 receptor regulates plasma clearance of exenatide following a three-month exposure in mice

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Background and aims: Exenatide is a glucagon-like peptide-1 receptor (GLP-1 R) agonist, which improves glycaemic control in patients with type 2 diabetes. The GLP-1R is expressed in multiple tissues and its activation is associated with numerous metabolic improvements including enhanced insulin secretion, slowing of gastric emptying, decreased food intake, and inhibition of hepatic steatosis. Based on current literature, it has not been established that the long-term glycaemic benefits of exenatide are exclusively mediated via activation of the GLP-1R. The aim of the present study was to examine the metabolic and pharmacokinetic effects of a supramaximal con-

tinuously infused dose of exenatide for 3 months in mice lacking a functional GLP-1R (GLP-1R KO).

Materials and methods: GLP-1R KO and wild-type (WT) control mice were fed a high fat diet (58% fat). Exenatide (30 nmol/kg/d) was continuously infused via osmotic mini-pumps for 3 months. HbA1c, blood glucose, plasma insulin, amylase, lipase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), body weight (BW), and food intake (FI) were measured. Oral glucose tolerance (OGTT) and gastric emptying were assessed at the end of the study. Terminal organ weights, hepatic lipid content, and plasma exenatide concentrations were measured. Results are presented as mean±SEM. The differences between groups were considered statistically significant for $P < 0.05$ (t-test).

Results: After 3 months treatment, no significant effects were observed with exenatide infusion on any metabolic endpoint in GLP-1R KO mice. In contrast, in WT mice exenatide significantly decreased vehicle (Veh)-corrected glucose by 25 ± 5 mg/dL, BW by $40 \pm 2\%$, and cumulative FI (maximally by $30 \pm 3\%$ at Week 2). Exenatide significantly reduced plasma insulin (0.5 ± 0.2 ng/mL) vs Veh (1.8 ± 0.5 ng/mL) and ALT (20 ± 4 U/L) vs Veh (47 ± 10 U/L). Exenatide reduced glucose excursion during an OGTT (AUC_{0-2h} (mg·h/dL) (642 ± 70) vs Veh (972 ± 93)). Hepatic lipid content was reduced by exenatide (g/g) (0.059 ± 0.004) in comparison to Veh (0.107 ± 0.010). Exenatide increased pancreatic (0.0097 ± 0.0004) and renal (0.0101 ± 0.0002) weight (g/g BW) vs respective Veh controls (0.0058 ± 0.0005 and 0.0081 ± 0.0005). Exenatide had no significant effect on HbA1c, amylase, lipase, gastric emptying, or liver weight under these experimental conditions. Intriguingly, plasma exenatide concentrations (450 ± 139 pg/mL) were approximately 8-fold higher in GLP-1R KO mice, than in WT controls (55 ± 20 pg/mL) ($P < 0.02$).

Conclusion: These results show for the first time that a functional GLP-1R is required for exenatide to exert its long-term effects in mice. The observed difference in plasma exenatide concentrations between wild type and GLP-1R KO mice suggests that GLP-1 receptors may play a significant functional role in the plasma and/or renal clearance of exenatide, and perhaps other related peptides. Furthermore, novel GLP-1 receptors may not be substantially involved in the chronic metabolic actions of exenatide in mice.

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Effects of different sweet preloads on incretin hormone responses, gastric emptying and postprandial glycaemia in healthy humans

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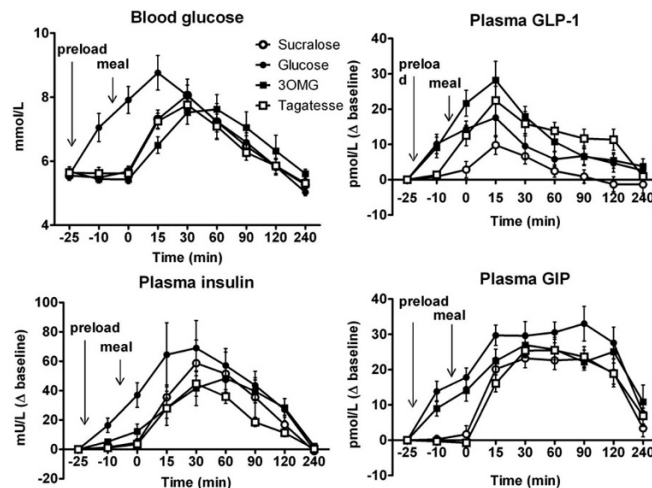
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Background and aims: Macronutrient 'preloads' taken in advance of a meal have the capacity to stimulate glucagon-like peptide 1 (GLP-1) from the distal, and glucose-dependent insulinotropic polypeptide (GIP) from the proximal gut, slow gastric emptying, and reduce postprandial glycaemic excursions in health and type 2 diabetes. These effects may potentially be signaled by sodium-glucose co-transporter 1 (SGLT1) and/or sweet taste receptors after sweet 'preloads'. We sought to compare the effects of four sweet 'preloads' on GIP and GLP-1 release, gastric emptying and glycaemia after a subsequent meal.

Materials and methods: 10 healthy subjects were studied on 4 days each in random order. On each day, subjects drank a 400mL 'preload' containing either 40g glucose, 40g Tagatose™ (a poorly absorbed mixture of tagatose and isomalt), 40g 3-O-methylglucose (3OMG; a non-metabolised substrate of SGLT1), or 60 mg sucralose (an artificial sweetener of equivalent sweetness) in water. 15 minutes later, they ate a mashed potato meal labelled with ¹³C octanoic acid. Blood glucose, total GLP-1 and GIP, insulin, and gastric emptying (breath test) were evaluated. Data are mean ± SEM.

Results: Both glucose and 3OMG stimulated GLP-1 and GIP release in advance of the meal ($P < 0.05$ for each), whereas Tagatose™ and sucralose did not. The GLP-1 response to the meal over 240 min (incremental area under the curve) was greater after glucose, 3OMG, and Tagatose™ than sucralose ($P < 0.01$ for each), and GLP-1 stimulation was later after Tagatose™. Blood glucose in the first 30 min after the meal (incremental area under the curve) was greatest after glucose ($P < 0.01$), but lower after 3OMG than sucralose ($P < 0.05$), while insulin was also greatest after glucose ($P < 0.05$). Gastric emptying was slower after 3OMG (half-emptying time 207 ± 17 min) and Tagatose™ (275 ± 39 min) than sucralose (165 ± 12 min, $P < 0.05$), and tended to be slower after glucose (245 ± 59 min) than sucralose ($P = 0.15$), without any difference between glucose, 3OMG and Tagatose™.

Conclusion: In healthy humans, SGLT-1 substrates, regardless of whether they are metabolised, stimulate GLP-1 and GIP and slow gastric emptying, whereas sweet taste per se has no effects. Poorly absorbed sweet tastants, which probably expose a greater length of gut to nutrient, result in later GLP-1 secretion and slowing of gastric emptying, but not GIP release. These observations can help optimise preloads for glycaemic control.



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Metabolic effects of sitagliptin in patients with type 2 diabetes

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Background and aims: Although the antihyperglycaemic effect of DPP4 inhibitors is thought to be mainly due to potentiation of insulin secretion via GLP-1, we aimed at assessing the integrated metabolic response to chronic DPP4 inhibitor treatment.

Materials and methods: We administered a mixed meal (MM) with 2 glucose (G) tracers (6,6D₂-G infused, D₁-G ingested) to 47 T2D patients (pts) (age=56±7 yrs [mean±SD], BMI=29.9±4.2 kg/m², HbA_{1c}=7.4±0.8%) and 7 age- and BMI-matched controls (C); pts were re-studied after 6 weeks of double-blind, randomised treatment with sitagliptin (sita, 100 mg/day, n=25) or placebo (plb, n=22).

Results: Changes from baseline (∅) in fasting G (-0.94 ± 0.89 vs 0.06 ± 1.78 mmol/L, $p < 0.01$) and G area-under-curve (AUC) (-594 ± 39 vs $+100 \pm 594$ mmol·L⁻¹·5h, $p < 0.0001$) were greater with sita than plb, respectively, in parallel with a lower appearance of oral G ($\Delta AUC = -356 \pm 917$ vs $+144 \pm 600$ μmol kg⁻¹·5h, $p = 0.01$), and a trend to greater suppression of endogenous G output ($\Delta AUC = -183 \pm 450$ vs -39 ± 411 μmol kg⁻¹·5h, $p = 0.1$). The plasma branched-chain aminoacid response was significantly lower after sita than plb (-8.76 ± 4.38 vs 3.96 ± 5.89 mmol·L⁻¹·5h, $p = 0.01$). Insulin sensitivity, significantly lower at baseline in pts vs C (271 ± 42 ml min⁻¹·m⁻² vs 389 ± 27 , $p < 0.0001$), improved by 11% after treatment ($\Delta = 30 \pm 30$ vs 3 ± 41 ml min⁻¹·m⁻², sita vs plb, $p < 0.01$). Baseline β-cell G sensitivity (β-GS), lower in pts vs C ($32[30]$ pmol min⁻¹·m⁻²·mM⁻¹ vs $98[115]$, $p = 0.0002$), improved with sita vs plb ($19[29]$ vs $5[21]$, $p = 0.01$). Glucagon and total GIP AUCs decreased ($p = 0.03$ and 0.01 , respectively) with sita vs plb, while total GLP-1 response was maintained. At baseline and follow-up, the G response to MM was matched by isoglycaemic (iso-G) IV G infusion. Sita vs plb increased β-GS with both MM and iso-G ($p = 0.01$ and $p = 0.002$, respectively), but the relative difference was unchanged from baseline.

Conclusion: Chronic sita treatment improves glycaemic control by lowering appearance of oral G and aminoacids, postprandial G release and glucagon response. Insulin sensitivity and β-cell G sensitivity also improve. While reversal of glucose toxicity may at least partly contribute to these findings, the results are consistent with potentiation of active GLP-1 effects on glucagon and β-cell function.

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Weight loss of 3% or more in a 12-month period is associated with glycaemic control in newly treated type 2 diabetes patients in the usual care setting

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Background and aims: Weight gain is reluctantly accepted as a trade-off for attaining glycemic control in patients with type 2 diabetes (T2DM), but this paradigm is poised to shift with the availability of new agents not associated with weight gain. This study evaluated the correlation between weight change and glycemic control in newly treated patients with T2DM in a usual care setting.

Materials and methods: Patients age 18+ years with T2DM newly treated with select antidiabetic agents including those associated with weight gain (sulfonylurea [SU], thiazolidinedione [TZD]) and those which are not (metformin [MET], DPP-4 inhibitor [DPP-4], or GLP-1 agonist [GLP-1]) as monotherapy were identified in a national electronic medical record (EMR) database from 1/1/2000 to 6/30/2010. Patients were required to have 410 days of EMR activity before and after the first antidiabetic prescription (index date). Outcomes included glycemic control (HbA1c <7% or ≥7%) and weight change (weight gain ≥3%, weight neutral [change <3%] and weight loss ≥3%) from baseline to 12 months. Logistic regression was used to identify the odds of attaining HbA1c goal with weight loss or weight gain vs. weight neutrality, controlling for baseline treatment and patient characteristics.

Results: The study included 28,290 patients; SU (6,283, 22.2%), TZD (2,978, 10.5%), MET (18,328, 64.8%), DPP-4 (456, 1.6%) and GLP-1 (245, 0.9%). Overall mean (±SD) age was 61 ±11.8 years; 46.4% were male. Mean (±SD) baseline weight and HbA1c were 95.3±22.7 kg and 7.5±1.6%, respectively; 25.5% had an HbA1c <7.0%. After 12 months, mean (±SD) weight change from baseline was -1.6±6.5 kg (p<0.001). Weight gain, weight loss, and weight neutrality were observed in 18.3%, 35.1% and 46.6% patients, respectively. The mean (±SD) reduction in HbA1c from baseline at 12 months was -0.7±1.6% (p<0.001). HbA1c goal attainment was achieved in 44.9% of all patients, and in 58.0% patients with weight loss, 36.6% patients with weight gain, and 38.3% patients who were weight neutral. Patients losing ≥3% of body weight were more likely to attain HbA1c goal relative to weight neutral patients (OR=2.7; 95% CI 2.55–2.87); those who gained weight were less likely to attain HbA1c goal (OR=0.77 95% CI 0.71–0.83). Baseline weight and the weight-effect properties of the baseline antidiabetic therapy currently did not predict HbA1c goal attainment.

Conclusion: Weight loss of ≥3% of body weight is a significant predictor of HbA1c goal attainment while baseline weight and weight-effect properties of the prescribed antidiabetic agents currently available are not. Future research will evaluate the associations between adherence and weight change, and their impact on glycemic outcomes.

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PS 067 Incretins: safety considerations for pancreas and kidney

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No worsening but improvement of exocrine pancreatic inflammation in spontaneously diabetic GK rats by DPP-IV inhibitor, vildagliptin

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Background and aims: Dipeptidyl-peptidase-IV (DPP-IV) inhibitor has emerged as a new type of diabetes treatment. There is a concern, however, for the effects on the exocrine pancreas. In one study, there was an increased occurrence of acute pancreatitis and ductal cell proliferation in human-IAPP transgenic rats treated with sitagliptin. The others found comparable episodes of pancreatitis in sitagliptin-treated subjects to those in healthy individuals. We therefore explored the long-term effects of DPP-IV inhibitor on the exocrine pancreas in GK rats, a model of non-obese type 2 diabetes.

Materials and methods: GK rats 4 weeks of age were given a DPP-IV inhibitor (vildagliptin 15mg/kg)(VG) twice a day by gavage for following 18 weeks. Control GK rats were given saline alone. Non-diabetic Wistar rats were divided into 2 groups in the same way. At end, all the animals were sacrificed and pancreases were subjected to the pathological evaluation with morphometric analysis.

Results: VG treatment did not influence on the body weight in either control or GK rats. While daily food intake was slightly reduced in GK rats, it was not the case in normal Wistar rats. There was a significant increase in serum amylase in GK rats compared to Wistar rats (719±14 vs 555±31, p<0.01) (mean±SE IU/L) and the value was normalized in VG-treated GK rats (501±42, p<0.01). VG treatment did not alter the values in control Wistar rats. On histology, GK rats showed scattered inflammatory foci in the parenchyma of exocrine pancreas, periductal areas and around small vessels. VG treatment lessened the severity of inflammation in GK rats and quantitation confirmed the decrease in the inflammatory foci. There was no significant difference in the rate of proliferating cells in duct or acinar cells by Ki67 staining between GK and Wistar rats. VG treatment did not influence on the proliferation activity in these cells. Acinar cells undergoing apoptosis detected by TUNEL staining (#/10hpf) were significantly increased in GK rats compared to Wistar rats (8.3±0.9 vs 2.7±0.7, p<0.01). VG treatment significantly suppressed the frequency of apoptotic cells (4.7±0.7, p<0.01).

Conclusion: Our present study demonstrated that VG treatment significantly suppressed inflammatory changes in the exocrine pancreas in GK rats without an increase in duct cell proliferation. It remains open to question whether the effects are specific to VG or GK rats, or in case common with other DPP-IV inhibitors.

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Long-term exposure of exenatide does not adversely affect exocrine pancreas structure and function in Diabetic Zucker Fatty (ZDF) rats

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Background and aims: Type 2 diabetes is a risk factor for the development of pancreatitis or pancreatic cancer. Previously, we have shown that exenatide did not exacerbate, but improved experimental pancreatitis in several rodent models. The aim of the present study was to characterize the effects of exenatide on exocrine pancreatic structure and function in ZDF rats treated chronically with exenatide at doses yielding plasma concentrations several fold higher than clinical exposure.

Materials and methods: Rats were dosed subcutaneously with exenatide (6, 40, 250 µg/kg/d split over 2 injections) or vehicle for 3 consecutive months. HbA1c, fasting serum lipase, amylase, glucose and insulin, body weight, and food consumption were measured monthly. Plasma exenatide concentrations were assessed at the beginning and end of the study. Pancreata were weighed and processed for histology. Morphometric analysis of duct cell proliferation and apoptosis was performed via immunostaining with cytokeratin (duct), Ki-67 (proliferation), and TUNEL (apoptosis). The same endpoints were measured after 1-month recovery in a subset of animals from each group.

Results are presented as mean \pm SD. The differences between groups were considered statistically significant when $P<0.05$ (ANOVA).

Results: As expected, exenatide improved glycaemic endpoints including a decrease in HbA_{1c} and glucose, and an increase in insulin and islet size, in comparison to vehicle. Exenatide had no effect on lipase. Treatment with exenatide was associated with a small, but significant increase in amylase concentrations (U/L) (exenatide 6 μ g/kg/d = 3,266 \pm 391, exenatide 40 μ g/kg/d = 3,517 \pm 404, exenatide 250 μ g/kg/d = 3,286 \pm 322) vs vehicle (2,706 \pm 289, $P<0.05$) at 3 months. There was no significant difference between all groups in amylase (U/L) at the end of the recovery period (exenatide 6 μ g/kg/d = 3,310 \pm 343, exenatide 40 μ g/kg/d = 3,376 \pm 330, exenatide 250 μ g/kg/d = 3,261 \pm 473, vehicle = 3,395 \pm 1,016). At the beginning of the study, the area under the exenatide plasma concentration-time curve (AUC_{0-8h}) ranged from 1 to 63 ng·h/mL and the maximal concentration (C_{max}) ranged from 1 to 43 ng/mL. These values were higher than AUC_{0-8h} and C_{max} seen with the highest recommended dose of exenatide in humans (20 μ g/d) by approximately 1- to 61-fold and 5- to 206 -fold, respectively. AUC_{0-8h} increased substantially after 3 months with the accumulation ratio ranging from 2.7 to 4.4. Importantly, exenatide improved survival of animals (100% survival in all treatment groups) in comparison to vehicle controls (60%) during the dosing period. There was no exenatide-related difference in absolute or relative pancreatic weight. Histological analysis revealed no changes in pancreatic exocrine tissue morphology in animals treated with exenatide. Mean ductal cell proliferation rate was low (<0.15%) and similar for all groups at the end of the treatment and after the recovery period. There was no significant difference in ductal cell apoptosis between all groups.

Conclusion: Long-term exposure to exenatide at plasma concentrations several fold higher than therapeutic levels in humans resulted in the expected metabolic benefits. Furthermore, exenatide improved animal survival and did not adversely affect exocrine pancreas structure and function in a rat model of type 2 diabetes.

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Comparison of vildagliptin with placebo in a 24-week study of 221 patients with type 2 diabetes and severe renal impairment (eGFR<30)

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Background and aims: Patients with type 2 diabetes (T2DM) have an increased prevalence of decreased kidney function, which progresses with increasing disease duration. In addition, pharmacokinetic data for vildagliptin, an orally effective dipeptidyl peptidase-4 (DPP-4) inhibitor, indicated an approximately 2-fold increased exposure in patients with severe renal impairment. It was therefore of interest to assess the safety and tolerability of vildagliptin as well as its efficacy in patients with T2DM and severe renal impairment.

Materials and methods: In a 24-week, multicenter, randomized, double-blind, parallel-group, placebo-controlled study, 221 patients with T2DM and severe renal impairment [estimated GFR (eGFR) <30 ml/min per 1.73 m² according to MDRD] were randomized to vildagliptin 50 mg qd (N=124) and placebo (N=97). Oral antidiabetic or insulin therapy present at enrollment (97.3%) was continued throughout the study. The primary endpoint was safety and tolerability; overall safety as well as specific safety areas of interest (hepatic, skin, edema and pancreatitis) are presented. The change in HbA_{1c} from baseline to study endpoint was analyzed by ANCOVA as a protocol-specified exploratory objective. The study also included 294 patients with moderate renal impairment, who are discussed in an accompanying abstract.

Results: Baseline and demographic characteristics of the randomized patients were representative of a T2DM population with severe renal impairment: mean eGFR = 21.5 ml/min per 1.73 m², mean age = 64.3 years (with 50.7% of patients \geq 65 years and 15.4% \geq 75 years), mean BMI = 30.0 kg/m², mean HbA_{1c} = 7.7% (with 69.2% \leq 8.0%) and mean disease duration = 18.1 years. 80.5% of patients were treated with insulin at study entry. Overall AEs, serious AEs, discontinuations due to AEs and deaths were reported with a comparable frequency in patients receiving vildagliptin and patients receiving placebo as shown in Table 1. There were no clinically relevant or statistically significant differences between treatment groups for hepatic-related AEs (0.8% with vildagliptin vs. 1.0% with placebo; $p>0.999$), skin-related AEs (2.4% vs. 6.2%; $p=0.185$), edema-related AEs (16.9% vs. 18.6%; $p=0.859$) and pancreatitis-related AEs (0% in both treatment groups). One patient each in the vildagliptin (0.8%) and placebo (1.1%) treatment groups had treatment-emergent, persistent ALT/AST elevations \geq 3xULN. Mean HbA_{1c} was reduced

in vildagliptin-treated patients by -0.88% from a baseline of 7.69%, with the placebo-subtracted reduction being -0.56% (95% CI -0.83 to -0.30, $p<0.0001$). **Conclusion:** Vildagliptin 50 mg qd is well tolerated and efficacious in T2DM patients with severe renal impairment.

Table 1. Overall safety in T2DM patients with severe renal impairment (Safety set)

	Vildagliptin 50 mg qd N=124 n (%)	Placebo N=97 n (%)
AEs	90 (72.6)	72 (74.2)
SAEs	23 (18.5)	20 (20.6)
Discontinuations due to AEs	11 (8.9)	6 (6.2)
Deaths	3 (2.4)	4 (4.1)

AE = adverse event; SAE = serious adverse event

Clinical Trial Registration Number: NCT00646542

Supported by: Novartis Pharmaceuticals

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Efficacy of vildagliptin therapy in combination with insulin in patients with type 2 diabetes and severe renal impairment

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Background and aims: Vildagliptin, a potent and selective DPP-4 inhibitor, improves islet function by increasing α - and β -cell responsiveness to glucose. In drug naive patients with T2DM there was a reduction in HbA_{1c} of 1.0 % from a baseline of 8.7% and a reduction of 0.5% from baseline of 7.3%. In patients where vildagliptin was added to metformin there was a 1.1% reduction in HbA_{1c} from baseline of 8.4% and a 0.7% reduction from a baseline of 7.8%. As most patients in this study had diabetes < 7 years, the question arises as to whether patients of longer duration of diabetes and with renal impairment, where beta cell sensitivity to glucose is often severely impaired, would be responsive to vildagliptin.

Materials and methods: The efficacy of vildagliptin in a subgroup of T2DM patients with severe renal impairment [estimated GFR (eGFR) < 30 ml/min per (1.73 m²) according to MDRD] inadequately controlled on insulin (HbA_{1c} of \geq 6.5 and \leq 10 %) from a large double blind placebo controlled study was analyzed. Vildagliptin 50 mg qd or matching placebo was added to the background therapy of insulin as monotherapy or in combination with oral anti-diabetic drugs (OADs) that remained unchanged through 24-week treatment. Efficacy was assessed as HbA_{1c} reductions at study endpoint from baseline and versus placebo using ANCOVA model. Safety and tolerability were also evaluated.

Results: 221 patients with severe renal impairment were randomized in the main trial. 178 of these patients received insulin treatment either alone (86%) or in combination with other OADs (mostly sulfonylureas). Mean age was 64 years, 48% were female, and the mean duration of diabetes was 19 years. Table 1 demonstrates that mean HbA_{1c} was reduced in vildagliptin-treated patients by -0.87% from a baseline of 7.7%, with the placebo-subtracted reduction being -0.59% ($p<0.0001$). Hypoglycemia overall and severe hypoglycemic events were reported at a similar rate in vildagliptin and placebo patients (19% vs 14% and 2% vs 3%, respectively) in spite of considerably greater HbA_{1c} reduction in the vildagliptin group. The overall incidences of AEs and SAEs were similar for vildagliptin and placebo (79% vs. 77% and 19% and 24%, respectively).

Conclusion: The mean HbA_{1c} reduction of 0.87% (0.59% decrease vs. placebo) from a baseline of 7.7% in insulin requiring patients with longstanding T2DM and severe renal impairment is consistent with the drops in HbA_{1c} that have been reported previously in vildagliptin-treated patients with much more recent onset of diabetes. Vildagliptin treatment in combination with insulin in patients with severe renal impairment appears to be as efficacious as in patients with less impairment in beta-cell function.

Table 1. ANCOVA results for change in HbA_{1c} (%) from baseline to rescue-censored endpoint treatment (full analysis set)

Treatment	Baseline	Adjusted Mean	Difference in adjusted mean change		
		change (SE)	(Vilda-Placebo)		
	n	Mean (SE)	Mean (SE)	(95% CI)	p-value
Vilda 50 mg qd	98	7.71 (0.10)	-0.87 (0.37)	-0.59 (0.15) (-0.88, -0.29)	0.0001*
Placebo	77	7.75 (0.12)	-0.29 (0.37)		

Clinical Trial Registration Number: NCT00646542

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Long-term efficacy and safety of linagliptin in patients with type 2 diabetes and severe renal impairment

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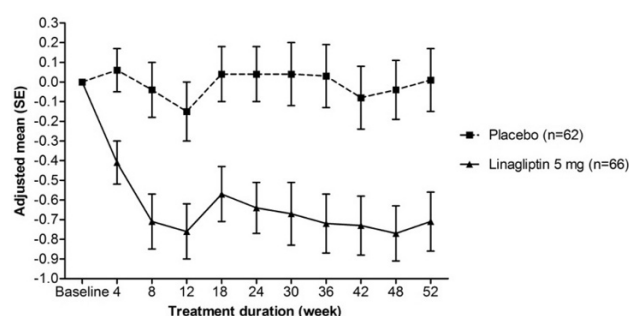
Background and aims: Renal impairment (RI) is a common complication of type 2 diabetes mellitus (T2DM). Linagliptin is a novel dipeptidyl peptidase-4 (DPP-4) inhibitor with unique pharmacological characteristics that allow the treatment of T2DM patients without dose adjustment for any degree of RI. This 52-week, randomised, double-blind, placebo (PBO)-controlled trial conducted at 53 centres in 6 countries evaluated the efficacy and safety of linagliptin in patients with T2DM and severe RI. The primary endpoint, HbA_{1c} change from baseline at week 12, was reported previously. Here we present the results of the full 52-week period.

Materials and methods: Patients with T2DM and severe RI (HbA_{1c} 7.0–10.0%; estimated glomerular filtration rate [eGFR] by MDRD formula <30 mL/min/1.73 m²) were randomised with equal probability to either linagliptin 5 mg once daily (n=68) or PBO (n=65), in addition to pre-existing glucose-lowering therapy. Background therapy doses could be adjusted only after week 12, according to glucose parameters.

Results: Mean values (±SD) for baseline age, HbA_{1c}, and eGFR were 64±10 years, 8.2±1.0%, and 24±7 mL/min/1.73 m², respectively. Diabetes duration was >5 years in 96% of patients and 80% were treated with insulin (alone or in combination). In the comparison vs PBO, the adjusted mean change in HbA_{1c} from baseline at week 52 was -0.72% (95% CI -1.03, -0.41; p<0.0001), confirming the superiority of linagliptin over PBO as demonstrated at week 12. HbA_{1c} values for the 12–52 week period showed that reductions were maintained throughout the study (Figure). Rates for any adverse event (AE) and serious AEs were similar with linagliptin (94.1% and 36.8%, respectively) and PBO (92.3% and 41.5%). The incidence of overall hypoglycaemia was higher with linagliptin (63.2%) than in the PBO group (49.2%); however, this difference was based on higher rates of mild, asymptomatic hypoglycaemic episodes and there was no difference in severe hypoglycaemic events (3 each). Renal function remained stable throughout the study in both treatment arms, and numbers of cardiovascular deaths in this high-risk population were similarly low (linagliptin, n=2 [2.9%]; PBO, n=3 [4.6%]).

Conclusion: Linagliptin, as a single dose not requiring adjustment, is an effective and durable treatment option for controlling blood glucose concentrations in T2DM patients with severe RI without an increased risk for clinically meaningful hypoglycaemia in this vulnerable patient population.

Adjusted HbA_{1c} (%) mean change from baseline over time



LS Mean ± SE, adjusted for HbA_{1c} baseline, creatinine clearance, and background glucose-lowering therapy

Clinical Trial Registration Number: NCT00800683

Supported by: Boehringer Ingelheim

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Reductions in HbA_{1c} and the incidence of hypoglycaemic episodes are not affected by renal impairment in patients with type 2 diabetes treated with liraglutide

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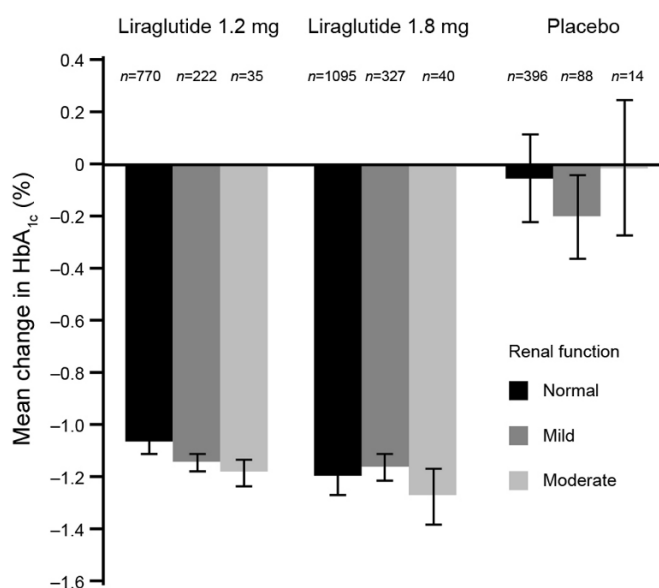
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Background and aims: Impaired renal function is often observed in people with type 2 diabetes (T2D). Clinical studies have previously shown that the once-daily human GLP-1 analogue, liraglutide, is not excreted in urine, but rather is completely degraded *in vivo* by DPP-4 and neutral endopeptidases. This meta-analysis investigated the efficacy and safety of liraglutide in patients with T2D to establish if treatment effectiveness is compromised in individuals with mild and moderate renal impairment (RI).

Materials and methods: A meta-analysis of 7 clinical trials in the liraglutide development programme was performed using data from 0–26 wks to assess changes in HbA_{1c} and serum creatinine (SC) from baseline and incidence of hypoglycaemia during liraglutide treatment (1.2 and 1.8 mg) in patients with normal renal function, compared with those with mild and moderate RI. Renal function was determined by estimated creatinine clearance [eCrCl] using the Cockcroft-Gault equation and defined as normal, eCrCl ≥ 90 mL/min; mild: eCrCl < 90 mL/min or moderately impaired, eCrCl < 60 mL/min; all corrected to a standard body surface area of 1.73 m². Changes in HbA_{1c} and SC were analysed using ANCOVA with trial treatment with or without metformin, and interaction between treatment (placebo, 1.2 and 1.8 mg liraglutide) and baseline eCrCl category as fixed effects and baseline HbA_{1c} or SC respectively as covariates.

Results: Over 26 wks, patients receiving either dose of liraglutide had greater HbA_{1c} reductions than patients receiving placebo. HbA_{1c} reductions were similar in patients with mild and moderate RI vs those with normal renal function (Fig), with no significant differences in HbA_{1c} reductions observed between groups. Even in patients with the greatest baseline RI (moderate group), reduction in HbA_{1c} with liraglutide was not compromised vs patients with normal renal function. The proportion of patients experiencing major hypoglycaemic episodes in this study was low (0–0.2%). Minor hypoglycaemic episodes appeared lower in patients with mild or moderate RI compared to those with normal renal function; a trend observed in all treatment groups. Changes in SC were small (-4.82 to 6.08 μmol/L). Interestingly, for moderate RI, SC was significantly improved in patients treated with 1.8 mg liraglutide (-4.82 μmol/L) vs placebo (6.08 μmol/L) (treatment difference: -10.90 μmol/L, p=0.0008).

Conclusion: In patients with T2D with concomitant mild to moderate RI, the glycaemic efficacy of liraglutide is similar to those with normal renal function. In addition, liraglutide treatment in patients with RI is not associated with an increased risk of hypoglycaemia. Liraglutide appears to be an effective and well-tolerated antidiabetic treatment in patients with T2D who also have mild or moderate RI.



Clinical Trial Registration Number: NCT00318422; NCT00318461; NCT00294723; NCT00333151; NCT00331851; NCT00518882; NCT00700817
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Changes in kidney function and renal adverse events in clinical trials comparing exenatide once weekly and exenatide twice daily

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Background and aims: Exenatide twice daily (EBID) 5 mcg or 10 mcg is a GLP-1 receptor agonist indicated in the European Union for the treatment of type 2 diabetes in combination with certain oral medications in patients with inadequate glycemic control. Data from 6 EBID, placebo-controlled trials do not show deleterious changes in kidney function in patients with near-normal estimated glomerular filtration rate (eGFR) treated with exenatide. This post-hoc analysis assessed changes in kidney function in patients treated with exenatide once weekly (EQW) 2 mg vs. EBID 10 mcg in 2 randomized, open-label clinical trials of 24–30 weeks.

Materials and methods: Subjects had sub-optimal glucose control (HbA_{1c} 7.1%–11.0%), had a BMI of 25–45 kg/m², and were treated with diet and exercise alone or with metformin, TZD, sulfonylurea, or combinations of these at baseline. Key exclusion criteria included a diagnosis of kidney disease or serum creatinine (SCr) > 1.4 mg/dL (female), > 1.6 mg/dL (male). Potential clinically important (PCI) renal function values for blood urea nitrogen (BUN) and SCr were defined as: BUN > 45 mg/dL; SCr > 1.6 mg/dL (male), > 1.4 mg/dL (female). Modified Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations were used to calculate the eGFR, with main analyses performed on subjects with baseline eGFR < 120 (N=518 using MDRD; N=532 using CKD-EPI).

Results: Baseline eGFR (mean±SD) was similar between groups (MDRD: EQW 79±17 mL/min, EBID 79±17 mL/min; CKD-EPI: EQW 86±17 mL/min, EBID 87±18 mL/min), as was the annualized change in eGFR from baseline to endpoint (LSMean±SE) (MDRD: EQW -6.1±2.4 mL/min, EBID -4.6±2.5 mL/min, p=0.674; CKD-EPI: EQW -5.2±2.4 mL/min, EBID -6.0±2.5 mL/min, p=0.816). There were no clinically meaningful changes from baseline to endpoint in BUN (LSMean±SE) for patients with eGFR < 120 by the MDRD equation (EQW -0.3±0.1 mg/dL, EBID -0.1±0.1 mg/dL) or for patients with eGFR < 120 by the CKD-EPI equation (EQW -0.2±0.1 mg/dL, EBID -0.1±0.1 mg/dL) or in SCr (MDRD: EQW 0.02±0.03 mg/dL, EBID 0.06±0.03 mg/dL; CKD-EPI: EQW 0.02±0.03, EBID 0.06±0.03 mg/dL) for either group. The proportion of subjects with elevated BUN (EQW 7%, EBID 4%, p=0.163) or elevated SCr (EQW 4%, EBID 4%, p=0.691) values at endpoint were similar between groups. The proportions of subjects with PCI elevated BUN (EQW 0%, EBID < 1%, p=0.312) or PCI elevated SCr (EQW 1%, EBID 1%, p=0.927)

values at endpoint were similar between groups. Comparison of the percentage of subjects with decreased or increased kidney function (defined as moving up or down CKD stage), based on the MDRD equation, showed no differences between EQW and EBID (decreased 14% vs 16%, no change 78% vs 77%, increased 8% vs 8%, p=0.951), comparing increased vs. decreased function between treatment groups. Similar results were obtained for EQW vs EBID, based on the CKD-EPI equation (decreased 15% vs 19%, no change 75% vs 72%, increased 10% vs 8%, p=0.202). There was no difference between groups in the incidence of renal treatment-emergent adverse events (TEAEs) (EQW 3%, EBID 3%, p=0.757).

Conclusion: This analysis does not show clinically meaningful differences between patients treated with EQW and EBID with regard to kidney function or renal TEAEs in subjects with normal or near-normal eGFR.

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DPP-4 inhibition: a new approach for the treatment of uraemic cardiomyopathy

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Background and aims: Uraemic cardiomyopathy contributes substantially to morbidity and mortality of patients with chronic kidney disease, which is in turn also a frequent complication of type 2 diabetes. Glucagon-like peptide-1 (GLP-1) may improve cardiac function and GLP-1 is mainly degraded by dipeptidyl peptidase-4 (DPP-4). Linagliptin is the only DPP-4 inhibitor that can be used clinically at all stages of renal insufficiency without dose adjustment. We investigated linagliptin in a rat model of chronic renal insufficiency (5/6 nephrectomy [5/6N]).

Materials and methods: Eight weeks after 5/6N or sham surgery, rats were treated orally with 3.3 mg/kg linagliptin or vehicle for 4 days, and, subsequently, plasma was sampled for 72 h for quantification of DPP-4 activity and GLP-1 levels. At the end of the study, heart tissue was harvested for mRNA analyses.

Results: 5/6N caused a significant (p < 0.001) decrease in GFR measured by creatinine clearance (sham: 2510±210 mL/24 h; 5/6N: 1665±104.3 mL/24 h) and increased cystatin C levels (sham: 700±35.7 ng/mL; 5/6N: 1434±77.6 ng/mL). DPP-4 activity was significantly reduced at all time points with no difference between sham or 5/6N animals. In contrast, active GLP-1 levels were significantly increased in 5/6N animals, as measured by the maximum plasma concentration (C_{max}; 5/6N: 6.36±2.58 pg/mL vs sham: 3.91±1.86 pg/mL; p < 0.001) and AUC_(0-72h) (5/6N: 201 pg·h/mL vs sham: 114 pg·h/mL; p < 0.001). The mRNA levels of cardiac fibrosis markers such as TGF-β, tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) and collagens 1a1 and 3a1 as well as markers of left ventricular dysfunction such as brain natriuretic peptide (BNP) were all significantly increased in 5/6N versus sham animals and consequently reduced or even normalized by linagliptin treatment (all p < 0.05; Table).

Conclusion: Linagliptin increased the AUC of GLP-1 approximately two-fold in a rat model of renal failure, and decreased gene expression of BNP, a marker of left ventricular dysfunction, as well as markers of cardiac fibrosis (TGF-β, TIMP-1, Col 1a1 and Col 3a1) in hearts of uraemic rats. These effects may support the use of linagliptin in uraemic cardiomyopathy; however, further studies are necessary to evaluate if the observed anti-fibrotic effects are caused by GLP-1 levels or are independent of the incretin pathway.

Ratio of changes in mRNA for treated versus control animals			
Marker	Sham	5/6N	5/6N + Linagliptin
TGF-β	0.83±0.02	0.93±0.05	0.80±0.10
TIMP-1	0.92±0.04	1.39±0.17	0.72±0.20
Col1a1	0.61±0.06	0.83±0.03	0.76±0.05
Col3a1	0.58±0.04	1.04±0.17	0.52±0.11
BNP	1.00±0.13	2.93±0.43	1.35±0.16

Supported by: Boehringer Ingelheim

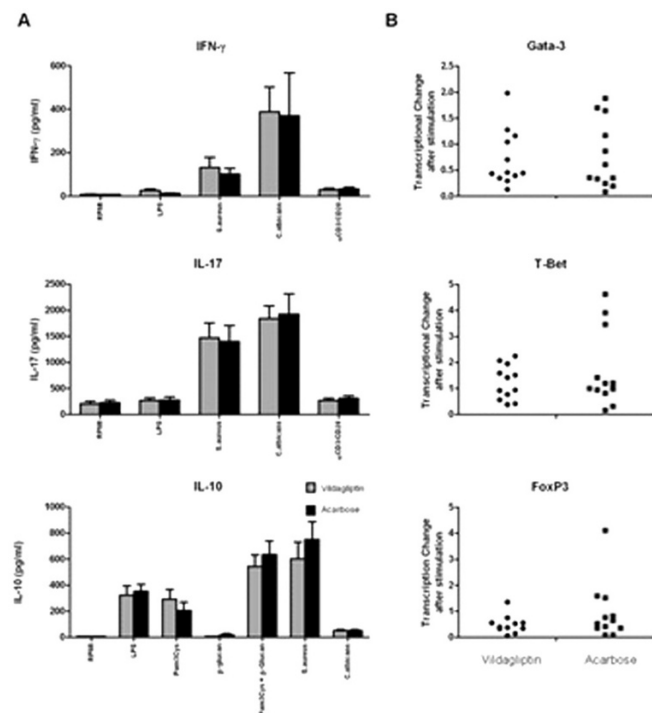
825

The dipeptidyl peptidase-4 inhibitor vildagliptin does not affect ex vivo cytokine response nor T-lymphocyte function in patients with type 2 diabetes mellitusP.C.M. van Poppel¹, M.S. Gresnigt², P. Smits³, M.G. Netea¹, C.J. Tack¹¹Department of Internal Medicine, ²Nijmegen Institute for Infection, Inflammation and Immunity (N4i), ³Department of Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Netherlands.

Background and aims: Dipeptidyl peptidase-4 (DPP-4), a key player in the degradation of incretin hormones and therefore a therapeutic target in type 2 diabetes, is also expressed on immune cells. DPP-4 inhibition may thus alter immune response, which could translate into susceptibility to infections. Some reports indeed suggest that the use of DPP-4 inhibitors is associated with an increased incidence of infections. The aim of this study, a substudy of a trial assessing the effect of vildagliptin on endothelial function, was to assess the effect of treatment with the DPP-4 inhibitor vildagliptin on both the innate immune production of cytokines and T-helper responses of patients with diabetes mellitus.

Materials and methods: Patients with type 2 diabetes on oral treatment (age 35–75 yrs, HbA1c < 8.0%) were treated with vildagliptin or acarbose for 4 weeks, in a randomized cross-over trial. At the end of each treatment period, peripheral blood mononuclear cells (PBMCs) were isolated and ex vivo stimulated with a broad spectrum of pattern recognition receptor agonists. In addition, T-cell function and activation was tested.

Results: The production of the proinflammatory cytokines IL-1 β , TNF- α and IL-6 by monocytes after 24h stimulation with LPS, Pam₃Cys, β -glucan, Pam₃Cys + β -glucan, heat killed *S. aureus* and *C. albicans* yeasts was not affected by vildagliptin. IL-1 β levels were 3884 \pm 389, 2052 \pm 474, 47 \pm 7, 4061 \pm 448, 1834 \pm 374 and 1425 \pm 184 pg/ml for vildagliptin and 4337 \pm 663, 1971 \pm 493, 40 \pm 1.3, 4027 \pm 462, 2003 \pm 370 and 1533 \pm 231 pg/ml for acarbose treatment, P=NS for all comparisons. Similar results were found for TNF- α and IL-6 production. Neither production of the T-cell cytokines IL-10, IFN- γ and IL-17 nor direct T-cell activation was affected by vildagliptin treatment (Fig 1). Finally, the relative increase of mRNA levels of T-cell lineage specific transcription factors T-bet, GATA-3 and FoxP3, did not differ between both treatment periods. (Fig 1) There were no carry over effects.



Conclusion: These data show that treatment of patients with type 2 diabetes mellitus with vildagliptin does not result in a significant modulation of both monocyte and T-cell-derived cytokine responses. These results, obtained in a relevant population, suggest that DPP-4 inhibition does not affect immune function. Figure 1. A Levels of the T-cell derived cytokines IFN- γ , Th17 and

IL-10 in the culture supernatants of PBMCs which stimulated after either vildagliptin (grey bars) or acarbose treatment (black bars). B Relative upregulation of mRNA transcription of T-cell lineage specific factors after stimulation with HK C. Albicans compared to unstimulated PBMCs.

Clinical Trial Registration Number: NCT01000688

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Trends in the characteristics of patients prescribed sitagliptin and other oral antihyperglycaemic agents over time in a large U.S. claims databaseT.D. Kou¹, K.G. Brodovicz¹, C.M. Alexander², E.A. O'Neill³, S. Engel⁴, C.J. Girman¹¹Epidemiology, Merck, North Wales, ²Global Medical Affairs, Merck, North Wales, ³Global Scientific and Medical Publications, Merck, Rahway, ⁴Clinical Research - Metabolism, Merck, Rahway, USA.

Background and aims: Differences in baseline demographic and clinical characteristics of patients initiating new therapy can introduce bias into comparative effectiveness/safety analyses. This study explored trends in the baseline characteristics of patients with type 2 diabetes (T2D) initiating sitagliptin, a DPP-4 inhibitor introduced in the U.S. in 2006, or other oral antihyperglycemic agents (OHA) between 2006 and April 2010 in a large U.S. insurance claims database.

Materials and methods: Using the United HealthCare I3 LabRx database, we identified a cohort of T2D patients with at least 1 new prescription for sitagliptin or other OHA between 1Jan2006 to 30Apr2010. Patients age 25–64 years receiving a new prescription in 2006–2007 were compared to patients receiving a new prescription in 2008–2010. Multivariate logistic regression analyses adjusting for age, sex, treatment complexity (monotherapy, dual, triple therapy), incident or prevalent T2D, comorbidities in prior 12 months, and time period were used to estimate odds ratios (OR) with 95% CI.

Results: Between 2006–2007, 11,550 patients filled a new prescription for sitagliptin and 106,122 filled a new prescription for another OHA. Between 2008–2010, 31,085 and 129,117 patients filled new prescriptions for sitagliptin or another OHA, respectively. Compared to new users of other OHAs, new sitagliptin users were older, more were male, less were newly diagnosed with T2D, and more were on combination therapy at the start of therapy (table). Comorbidities were consistently higher for new sitagliptin users across most of the comorbidities assessed, including blindness/macular edema/retinopathy, heart failure, hypertension, myocardial infarction, neuropathy, peripheral vascular disease, proteinuria, and renal failure. These differences were consistent across periods. After adjustment for covariates in the table, new sitagliptin users remained older and more likely to use combination therapy. While age and combination therapy were strong confounders, the prevalence of several comorbidities remained significantly higher in new sitagliptin users at the start of therapy (blindness/macular edema/retinopathy, hypertension, proteinuria).

Conclusion: Based on this analysis of a large cohort in a U.S. claims database, new sitagliptin users consistently had a higher proportion of several important comorbidities compared to new users of other OHAs. These differences in baseline characteristics were observed not only in the first 2 years post-approval but persisted 2–4 years post-approval. This has significant implications for pharmacoepidemiology observational studies in electronic medical record or claims databases and supports the need for control of channeling bias in such studies.

*Adjusted for all variables in the table

	Jan 2006 - Dec 2007			Jan 2008 - Apr 2010		
Baseline Characteristics	Other OHAs n=106,122	Sitagliptin n=11,550	Adjusted OR*(95% CI)	Other OHAs n=129,117	Sitagliptin n=31,085	Adjusted OR*(95% CI)
Age(yrs)	11.7%	7.0%	ref	11.8%	8.0%	ref
25-39	26.1%	23.3%	1.33	25.6%	24.4%	1.25
40-49	42.6%	45.7%	(1.21,1.45)	41.9%	44.4%	(1.19,1.31)
50-59	19.7%	24.1%	1.50	20.7%	23.1%	1.31
60-64			(1.38,1.64)			(1.25,1.37)
			1.60			1.34
			(1.45,1.75)			(1.27,1.40)
Sex	55.7%	60.0%	ref	55.5%	58.6%	ref
Male	44.3%	40.0%	0.97	45.0%	41.1%	1.01
Female			(0.93,1.02)			(0.98,1.03)
New T2D	38.7%	29.3%	ref	39.7%	26.8%	ref
Existing T2D	61.3%	70.7%	0.81	60.3%	73.2%	1.07
			(0.77,0.85)			(1.04,1.10)
Monotherapy	72.2%	25.5%	ref	76.2%	32.7%	ref
Dual	22.7%	39.3%	5.69	20.2%	39.5%	4.70
Triple	5.1%	35.2%	(5.40,5.99)	3.5%	27.8%	(4.58,4.82)
			24.99(23.43,26.67)			18.81(18.15, 19.49)
Blindness/macular edema/retinopathy	7.3%	11.3%	1.03	7.4%	10.1%	1.01(1.0,1.03)
			(1.01,1.05)			
Heart failure	1.7%	2.3%	1.01	1.6%	2.1%	1.02(1.0,1.03)
			(0.98,1.04)			
Hypertension	64.7%	73.5%	1.04	65.3%	71.1%	1.04
			(1.03,1.05)			(1.03,1.04)
Myocardial infarction	11.3%	15.0%	1.00	10.0%	12.3%	1.00
			(0.99,1.01)			(0.99,1.00)
Neuropathy	6.1%	7.5%	1.02	5.9%	6.9%	1.02
Peripheral vascular disease	3.0%	4.4%	(1.00,1.04)	2.9%	3.7%	(1.01,1.03)
			1.02			1.01
			(1.00,1.04)			(1.00,1.02)
Proteinuria	1.9%	2.9%	1.08	1.8%	2.7%	1.06
Renal failure	1.9%	3.3%	(1.02,1.14)	2.2%	3.2%	(1.03,1.10)
			1.00			1.00
			(0.99,1.01)			(1.00,1.00)

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Saxagliptin vs glipizide as add-on therapy to metformin in patients with type 2 diabetes: a 2-year assessment of HbA_{1c}, hypoglycaemia, and weight gain in a randomised, double-blind study

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Background and aims: Hypoglycemia and fears of hypoglycemia hinder glycaemic goal achievement and may delay intensification of antidiabetic therapy. Hypoglycemia and weight gain are known risks of sulfonylurea therapy, and its use may impede the safe and effective management of type 2 diabetes (T2D). In clinical practice, it is important to achieve glycaemic goals without weight gain or episodes of hypoglycemia. The dipeptidyl peptidase-4 inhibitor saxagliptin (SAXA) potentiates insulin secretion in a glucose-dependent manner and is associated with a low incidence of hypoglycemia and is weight neutral. A post hoc analysis was conducted to explore the difference in hypoglycemic events and weight gain in patients with T2D reaching HbA_{1c} goals after treatment with SAXA or glipizide (GLIP) as add-on to metformin (MET).

Materials and methods: In a multicenter, randomized, double-blind, parallel-group study, adults with HbA_{1c} >6.5% and ≤10% were given SAXA 5 mg/d (n=428) or GLIP titrated from 5–20 mg/d (n=430) as an add-on to MET for 52 weeks. Patients were treated for an additional 52 weeks in a prospective, blinded extension period, yielding a total observation period of 104 weeks. Assessment at 104 weeks showed that SAXA was similar to GLIP in lowering HbA_{1c} when added to MET and was well tolerated. The proportion of patients with adverse events (excluding hypoglycemia) in the 2 treatment groups was similar. A post hoc analysis examined 1) the difference in the frequency of hypoglycemic events between the SAXA and GLIP groups by baseline HbA_{1c} categorical groupings using a Kaplan-Meier analysis and 2) the proportion of patients with baseline HbA_{1c} ≥7% who had HbA_{1c} <7% observed at week 104 without hypoglycemia or weight gain of ≥2% (last observation carried forward).

Results: Fewer SAXA-treated patients (3.5%) than GLIP-treated patients (38.4%) had ≥1 hypoglycemic event through week 104. Hypoglycemic events occurred earlier and more frequently in patients treated with GLIP compared with SAXA in all subgroups of baseline HbA_{1c}. In both treatment groups, the number of patients with hypoglycemic events was higher with progression from higher to lower baseline HbA_{1c}. No SAXA-treated patients who had baseline HbA_{1c} ≥9% had a hypoglycemic event. More patients treated with SAXA had no weight gain during treatment (Table). Although approximately the same proportion of patients in each treatment group achieved HbA_{1c} <7%, more patients treated with SAXA vs GLIP did so without experiencing hypoglycemia, with weight gain <2%, or both (Table).

Conclusion: Regardless of baseline HbA_{1c}, fewer patients experience hypoglycemic events with the addition of SAXA to MET compared with the addition of GLIP. The difference is more pronounced at lower baseline HbA_{1c} levels, highlighting the safety of SAXA in patients with lower baseline HbA_{1c} levels. Compared with addition of GLIP to MET, addition of SAXA to MET resulted in more patients achieving glycaemic control without hypoglycemia and weight gain over a 2-year period.

Endpoint at Week 104	SAXA 5 mg + MET n=428*	GLIP + MET n=430*
Patients included in analysis [†] , n	324	322
Weight gain <2%, n (%)	266 (82.1)	190 (59.0)
HbA _{1c} <7%, n (%)	75 (23.1)	73 (22.7)
HbA _{1c} <7% and no hypoglycemia, n (%)	72 (22.2)	43 (13.4)
HbA _{1c} <7% and weight gain <2%, n (%)	65 (20.1)	47 (14.6)
HbA _{1c} <7%, no hypoglycemia, and weight gain <2%, n (%)	63 (19.4)	28 (8.7)

*Total number of patients in each group.

[†]Patients in this analysis had baseline HbA_{1c} ≥7% and ≥1 post-baseline weight measurement; patients were required to have an HbA_{1c} value <7% in the week 104 analysis window in order to have achieved goal; weight data were last observation carried forward.

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Effects of saxagliptin added to sub-maximal doses of metformin compared with dose escalation of metformin in type 2 diabetes: results from the PROMPT study

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Background and aims: High doses of metformin (MET) are often associated with an increased incidence of gastrointestinal (GI) symptoms which may lead to poor patient (pt) compliance and physician reluctance to increase the dose. The PROMPT study aimed to compare the efficacy and tolerability of 2 different treatment intensification strategies, either adding saxagliptin (SAXA) or uptitrating MET monotherapy (MET-UP) in pts with type 2 diabetes (T2D) and inadequate glycaemic control on a sub-maximal dose of MET 1.5 g/d.

Materials and methods: In this pan-European (Belgium, France, Germany, Italy, Spain, Turkey, UK), double-blind, parallel-group study, pts with T2D on MET monotherapy (1.5–1.7 g/d; HbA_{1c} ≥7–≤10% at study entry) were randomised to receive for 24 weeks, on a standardised, fixed dose of MET 1.5 g/d, either add-on SAXA 5 mg/d (SAXA+MET) or a 2-step MET uptitration, to a maximum dose of 2.5 g/d (MET-UP). GI symptoms were assessed using the 5-item validated Digestive Health Status Index (DHSI). Pts previously intolerant or non-compliant to MET >1.5 g/d were excluded.

Results: 286 pts ≥18 y were randomised. Mean baseline HbA_{1c} values (±SD) were 7.7±0.8% and 7.8±0.8% in the SAXA+MET and MET-UP groups, respectively. After 24 weeks, reductions from baseline in HbA_{1c} were (adjusted mean) -0.47% for SAXA+MET and -0.38% for MET-UP; mean (95% CI) difference in treatment effect, -0.10% (-0.26%, 0.07%); p=0.26. Discontinuations due to lack of efficacy were 10.9% for SAXA+MET vs 16.5% for MET-UP. The overall incidence of adverse events (AEs) was 51.0% for SAXA+MET vs 43.9% for MET-UP. The incidence of any hypoglycaemic event was 6.8% in the SAXA+MET group vs 2.2% in the MET-UP group, although the number of pts with a confirmed hypoglycaemic event (<3 mM) did not differ between the 2 groups. The 2 most common (>5%) AEs were diarrhoea (6.1% vs 12.2%) and nasopharyngitis (5.4% vs 1.4%) in the SAXA+MET and MET-UP groups, respectively. Exploratory analyses of the DHSI questionnaire showed differences (mean ±SE) in the diarrhoea predominant score (+0.8±1.7 vs +7.9±1.7; p=0.003) and dysmotility score (-0.5±0.8 vs +1.9±0.8; p=0.03) in the SAXA+MET and MET-UP groups, respectively, demonstrating the GI inconvenience of uptitrating MET in this pt group. No significant differences were reported for the other 3 DHSI items. Mean body weight (±SD) decreased in both treatment groups; -1.1±3.5 kg in pts treated with SAXA+MET and -1.6±2.7 kg in the MET-UP group.

Conclusion: In pts with T2D inadequately controlled on sub-maximal doses of MET and with no previously reported MET-related tolerability issues, addition of SAXA 5 mg/d to MET monotherapy was well tolerated. The HbA_{1c} reductions observed in the SAXA+MET and the MET-UP groups were not statistically different but there was an increase in GI side effects with the MET-UP intensification strategy. These data suggest that the SAXA add-on treatment strategy is a suitable alternative to MET uptitration in pts with T2D not well controlled on sub-maximal MET monotherapy.

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Clinical characteristics and sustained glycaemic control: a 76-week, randomised, double-blind study of saxagliptin + metformin in treatment-naïve patients with type 2 diabetes

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Background and aims: The efficacy of the dipeptidyl peptidase-4 inhibitor saxagliptin (SAXA) was evaluated as initial therapy with metformin (MET) in treatment-naïve patients with type 2 diabetes (T2D). After 24 weeks of therapy, significantly more patients achieved glycaemic response (HbA_{1c} <7%) with SAXA + MET combination therapy (SAXA 5 mg + MET, 60.3%; SAXA 10 mg + MET, 59.7%; P<0.0001) than with SAXA 10 mg alone (32.2%) or

MET alone (41.1%). SAXA was well tolerated. Similar proportions of patients in each group had adverse events. Durability of glycemic response is important for long-term T2D treatment. We conducted a post hoc analysis to 1) compare sustained glycemic response (SGR, defined as $HbA_{1c} < 7\%$ observed at both weeks 24 and 76; patients not meeting criteria at either time were treated as failures) with SAXA + MET vs MET monotherapy; 2) identify relationships between achieving SGR and key baseline (BL) characteristics; 3) determine whether there was a differential effect for SAXA + MET vs MET monotherapy.

Materials and methods: In this phase 3, double-blind, parallel-group trial, 1306 patients with T2D inadequately controlled (HbA_{1c} 8–12%) with diet and exercise were randomly assigned 1:1:1 to receive oral SAXA 5 mg + MET, SAXA 10 mg + MET, SAXA 10 mg, or MET. Differences in the proportion of patients with SGR were presented as percentages with exact 95% CI. Logistic regression models included treatment, select BL covariates (Table), and treatment*BL covariate interaction.

Results: BL characteristics were generally well balanced among randomized groups (mean age, 52 y; T2D duration, 1.7 y; BL HbA_{1c} , 9.5%). A greater proportion of patients treated with SAXA 5 mg + MET (38.1%) and SAXA 10 mg + MET (36.8%) than with MET alone (22.0%) had SGR (difference SAXA 5 mg + MET vs MET: 16.2%, 95% CI, 9.1–23.1; difference SAXA 10 mg + MET vs MET: 14.9%, 95% CI, 7.9–21.8). The greater proportion of patients achieving SGR with SAXA + MET than with MET alone was not differentially affected by demographic or BL patient characteristics. For BL clinical characteristics, the chance of achieving SGR in all treatment groups was improved with increased BL homeostasis model assessment 2 β -cell function (HOMA-2B), insulin AUC, C-peptide AUC, or age; reduced by increased BL HbA_{1c} or postprandial glucose AUC; numerically reduced in women; and not clearly associated with BMI (Table). Increased glucagon AUC and duration of T2D reduced the chance of SGR with MET, but not with SAXA + MET.

Conclusion: Initial therapy with SAXA + MET consistently resulted in more patients achieving SGR vs MET monotherapy over a 76-week period in treatment-naïve adults with inadequately controlled T2D, regardless of BL characteristics. The BL clinical characteristics associated with SGR were generally similar among treatment subgroups. These results suggest that SGR may be more likely when therapy is begun at a less severe stage of deterioration in glycemic control.

Baseline or Demographic Characteristic	Odds Ratio for Achieving SGR (95% CI)		
	SAXA 5 mg + MET n=320	SAXA 10 mg + MET n=323	MET n=328
10-unit increase in HOMA-2B	1.17 (1.08–1.27)	1.25 (1.15–1.35)	1.18 (1.14–1.23)
1000-unit increase in postprandial insulin AUC	1.16 (1.06–1.27)	1.11 (1.01–1.22)	1.12 (1.07–1.18)
100-unit increase in postprandial C-peptide AUC	1.16 (1.06–1.26)	1.10 (1.01–1.20)	1.14 (1.09–1.19)
10-y increase in age	1.17 (0.91–1.50)	1.30 (1.02–1.67)	1.09 (0.96–1.23)
1% increase in HbA_{1c}	0.63 (0.50–0.79)	0.64 (0.51–0.80)	0.66 (0.59–0.74)
1000-unit increase in PPG AUC	0.95 (0.93–0.98)	0.96 (0.94–0.99)	0.95 (0.94–0.97)
1000-unit increase in postprandial glucagon AUC	0.97 (0.88–1.07)	0.99 (0.90–1.09)	0.93 (0.89–0.98)
1-y increase in duration of diabetes	0.96 (0.85–1.08)	0.98 (0.87–1.11)	0.92 (0.87–0.98)
Women vs men	0.94 (0.56–1.59)	0.72 (0.43–1.21)	0.88 (0.67–1.14)
1-unit increase in BMI	1.03 (0.97–1.09)	0.96 (0.91–1.01)	1.00 (0.98–1.03)

AUC=area under the curve; BMI=body mass index; HOMA-2B=homeostasis model assessment 2 β -cell function; MET=metformin; PPG=postprandial glucose; SAXA=saxagliptin; SGR=sustained glycemic response ($HbA_{1c} < 7\%$ at weeks 24 and 76).

Clinical Trial Registration Number: NCT00327015

Supported by: BMS and AZ

830

Metformin/saxagliptin combination therapy can replace insulin therapy in patients with type 2 diabetes: interim results from the SAXAswitch study

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Background and aims: Type 2 diabetes is a complex disease with various phenotypes, as patients suffer from vascular and metabolic insulin resist-

ance and β -cell dysfunction with different degrees of severity. We previously showed that patients on insulin treatment with predominant insulin resistance can be switched back to oral treatment with insulin sensitizers. In this study, we investigate whether in patients with residual β -cell function (low proinsulin and normal C-peptide levels) an oral combination treatment with saxagliptin and metformin (Saxa+Met) can also replace an existing insulin therapy

Materials and methods: The participants in this study had to be on insulin therapy for more than one year and good control ($HbA_{1c} < 7.5\%$). They were subjected to a 1:2 randomization to stay on insulin (n = 37; 19 women, 18 men, age: 63 ± 8 yrs., disease duration: 11.0 ± 5.3 yrs., BMI: 33.3 ± 6.4 kg/m², HbA_{1c} : 6.8 ± 0.5 %) or to switch to an oral combination therapy with 2x 850 mg of metformin and 5 mg saxagliptin (n = 72, 31 women, 41 men, age: 62 ± 9 yrs., disease duration: 11.9 ± 7.4 yrs., BMI: 33.9 ± 5.6 kg/m², HbA_{1c} : 6.8 ± 0.5 %) for an observation period of 6 months. In case of worsening of glycemic control (HbA_{1c} increase > 0.4 %), pioglitazone was provided as first rescue drug (+Pio), and insulin glargine served as 2nd rescue drug (+Glarg).

Results: This completer analysis was performed with the 92/109 patients (84 %), who had completed the trial with stable glycemic control. Only 3/37 patients had dropped out in the insulin group for protocol violations (= 92 % completers,) and 14/72 patients stopped the study prematurely in the oral treatment group for reasons of HbA_{1c} -increase, adverse events, or patient decision (81 % completers). From the 58 completers on oral therapy, 21 were finally on Saxa+Met (36 %, HbA_{1c} at endpoint: 6.6 ± 0.4 %, Change in BMI: -1.2 ± 0.3 kg/m²), 19 were on Saxa+Met+Pio (33 %, 6.8 ± 0.5 %, -1.1 ± 0.2 kg/m²), and 18 were on Saxa+Met+Pio+Glarg (31 %, 7.2 ± 0.6 %, Change in BMI: -1.9 ± 0.4 kg/m²). There were no differences regarding type and severity of adverse events between the two treatment arms.

Conclusion: A combination of modern oral drugs addressing the underlying disease pathophysiology may allow a successful switch back from insulin treatment to oral combination therapy in patients with residual β -cell function. Further analysis will now be performed to identify laboratory biomarkers able to indicate the severity of β -cell dysfunction and with predictive value for such a successful switch from insulin to oral anti-diabetic drug combinations including saxagliptin.

Clinical Trial Registration Number: EUDRA-CT 2009-016745-25

Supported by: Astra-Zeneca + Bristol Myers Squibb

831

Efficacy and safety of 5 mg daily dosing regimens with linagliptin in patients with type 2 diabetes inadequately controlled on metformin

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Background and aims: Glycaemic control in patients with type 2 diabetes mellitus (T2DM) can be difficult using monotherapies such as metformin as target blood glucose levels are often not achieved. Addition of further agents, such as the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin, to existing therapy has been shown to improve glycaemic control. This study was conducted to evaluate whether twice-daily (bid) dosing with linagliptin 2.5 mg would achieve comparable efficacy and safety to once-daily (qd) linagliptin 5 mg (ie, the same total daily dose) when given on top of metformin bid in patients with inadequate glycaemic control.

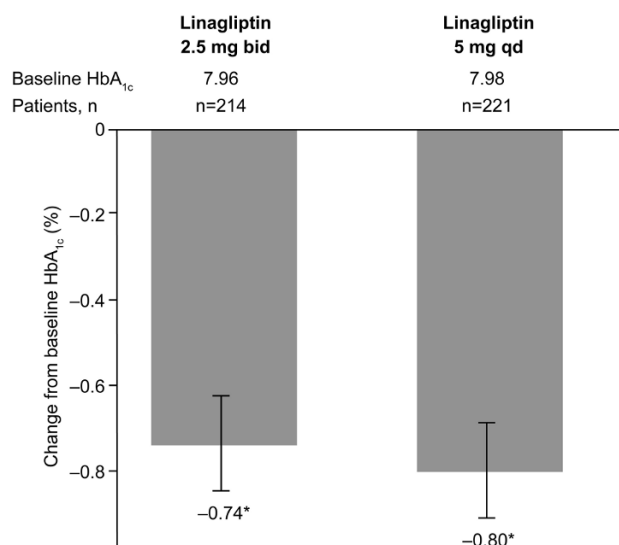
Materials and methods: Individuals (HbA_{1c} 7–10%) were randomized (5:5:1) to receive linagliptin 2.5 mg bid or 5 mg qd, or placebo for 12 weeks in addition to background metformin bid (≥ 1500 mg/day or maximally tolerated dose). The primary endpoint was change from baseline in HbA_{1c} after 12 weeks, while secondary endpoints included HbA_{1c} lowering of $\geq 0.5\%$, and change from baseline in fasting plasma glucose (FPG) after 12 weeks. The efficacy of linagliptin 2.5 mg bid versus 5 mg qd was compared using a pre-specified non-inferiority margin (95% CI within +0.35% HbA_{1c}).

Results: In total, 491 patients with a mean baseline HbA_{1c} 8.0% were randomized. After 12 weeks, linagliptin 2.5 mg bid and 5 mg qd significantly reduced HbA_{1c} (placebo-adjusted changes -0.74% [95% CI -0.97 , -0.52] and -0.80% [95% CI -1.02 , -0.58]; both $p < 0.0001$; Figure). Efficacy was comparable for the two linagliptin regimens with a difference in HbA_{1c} (0.06% [95% CI -0.07 , 0.19]) that was within the predefined noninferiority margin. FPG was also reduced versus placebo (2.5 mg bid -13.7 mg/dL [95% CI -22.7 , -4.7]; $p = 0.0029$; 5 mg qd -17.8 mg/dL [95% CI -26.7 , -8.8]; $p < 0.0001$). The

proportion of patients achieving HbA_{1c} reductions of $\geq 0.5\%$ was 55.1% with linagliptin 2.5 mg bid, 59.7% with 5 mg qd and 16.3% with placebo. Rates of adverse events (AEs) were similar with linagliptin 2.5 mg bid, 5 mg qd and placebo (43.0%, 34.8% and 38.6%, respectively). Most AEs were mild-to-moderate in intensity and few were deemed related to study medication in any group. Hypoglycaemic events were rare (3.1% for linagliptin 2.5 mg bid, 0.9% for 5 mg qd and 2.3% for placebo) and there were no cases of severe hypoglycaemia.

Conclusion: Both 5 mg daily dosing regimens of linagliptin provided meaningful efficacy in patients inadequately controlled with metformin monotherapy. Linagliptin achieved HbA_{1c} reductions of up to 0.8% after 12 weeks of treatment in this study.

Placebo-corrected adjusted mean (SE) changes in HbA_{1c} from baseline after 12 weeks with linagliptin 2.5 mg bid or 5 mg qd (FAS [LOCF])



*P<0.0001 vs placebo

The 12-week change from baseline in the placebo group (n=43) was +0.28% (SE 0.11) from a mean baseline HbA_{1c} 7.92%

Clinical Trial Registration Number: NCT01012037

Supported by: Boehringer Ingelheim

832

Linagliptin improves glycaemic control independently of diabetes duration and insulin resistance in patients with type 2 diabetes

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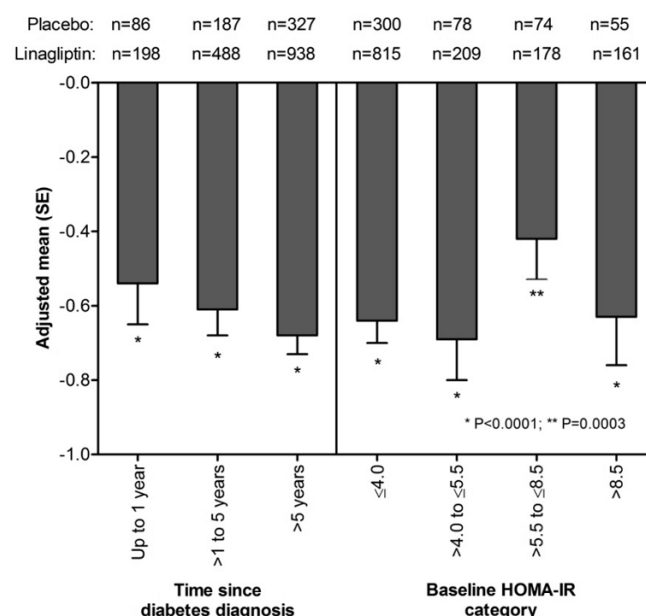
Background and aims: Three randomised, double-blinded, placebo-controlled, phase 3 trials for the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin examined safety and efficacy of glycaemic control as monotherapy, as add-on to metformin, or as add-on to metformin + sulfonylurea (SU) in patients with type 2 diabetes mellitus (T2DM). Identical endpoints, study duration, linagliptin dosing, and a large cohort size (N=2,258) facilitated subgroup analyses using the pooled dataset. Given the need for evaluation of the safety and efficacy of new antidiabetic agents on a background of other medications and patient comorbidities, we analysed pooled patient data to evaluate the effect of key patient characteristics on the safety and efficacy of linagliptin. Some research studies have shown a reduced treatment response in patients with longer diabetes duration and increased insulin resistance (IR), thus we determined the response to linagliptin treatment in patients with different diabetes durations and different stages of IR.

Materials and methods: The primary efficacy outcome in all three pooled studies was mean change from baseline in HbA_{1c} at 24 weeks. The incidence of any adverse events (AE) was recorded. Patients were categorised according to the years of diabetes duration: ≤ 1 , >1 -5, and >5 years. Furthermore, patients were categorised according to IR (referred to as HOMA-IR categories): ≤ 4 , >4 - ≤ 5.5 , >5.5 - ≤ 8.5 , >8.5 mU/L*mmol/L.

Results: The mean (\pm SD) patient age and baseline BMI were 57 ± 10 years and 29.0 ± 4.9 kg/m², respectively. Patients were predominantly white (58%) and Asian (42%), with an equal gender distribution. Mean disease duration was up to 1 year in 13% of patients, >1 -5 years in 30% of patients, and >5 years in 57% of patients. Sixty percent of patients were in the HOMA-IR category of ≤ 4.0 mU/L*mmol/L, 15% in the category of >4.0 - ≤ 5.5 mU/L*mmol/L, 13% in the category of >5.5 - ≤ 8.5 mU/L*mmol/L, and 12% in the category of >8.5 mU/L*mmol/L. Forty percent of patients were overweight (mean BMI 27.5 ± 1.4 kg/m²), and 38% were obese (mean BMI 34.1 ± 3.0 kg/m²). Mean baseline HbA_{1c} (\pm SD) and HOMA-IR were $8.1 \pm 0.8\%$ and 4.7 ± 5.3 mU/L*mmol/L, respectively. Significant reductions in HbA_{1c} levels after 24 weeks in the 3 groups of diabetes duration and 4 groups of baseline IR are shown in the figure. The overall hypoglycaemic event rate with linagliptin as monotherapy and add-on to metformin therapy was very low ($\leq 1.0\%$). A higher rate of hypoglycaemic events with linagliptin only occurred in the study that used a background therapy with metformin and SU; this was expected due to the combination with SU.

Conclusion: Treatment with linagliptin provided clinically meaningful HbA_{1c} reductions in patients with T2DM independent of the diabetes duration and the degree of IR, with a safety profile comparable to placebo. The reductions in HbA_{1c} were consistent with results from the primary phase 3 trials.

HbA_{1c} (%) change from baseline at week 24 with linagliptin (difference from placebo)



* P<0.0001; ** P=0.0003

Supported by: Boehringer Ingelheim

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Achieving the composite end point of HbA_{1c} <7%, no hypos, and no weight gain: comparison between vildagliptin and glimepiride after 2 years of treatment

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Background and aims: It has previously been shown that, as add-on to metformin vildagliptin has similar efficacy on HbA_{1c} as glimepiride after 2 years of treatment but is associated with markedly reduced hypoglycemia risk and no weight gain. We performed a post-hoc analysis, where we investigated whether there were differences in a clinically relevant composite end point of HbA_{1c} <7%, no hypos (symptoms and plasma glucose <3.1 mmol/L), and no significant weight gain ($<3\%$) in relationship to duration of diabetes and/or the age of the patients.

Materials and methods: In this study, 3118 patients were randomized (vildagliptin, n = 1562; glimepiride, n = 1556): the overall baseline HbA_{1c} was 7.3%. After two years, a similar proportion of patients reached HbA_{1c} <7% (36.9 and 38.3%, respectively with vildagliptin and glimepiride), but with vildagliptin more patients reached this target without hypoglycemia (36.0% vs. 28.8%; p = 0.004).

Results: The data from subjects with baseline HbA_{1c} >7% were analyzed (vildagliptin, HbA_{1c} = 7.6±0.52, n = 1036; glimepiride, HbA_{1c} = 7.6±0.55, n = 980) and are shown in table.

Conclusion: In patients with type 2 diabetes mellitus and baseline HbA_{1c} in the 7 - 8.5 % range, vildagliptin shows a better clinical benefit - as defined by the composite end point of HbA_{1c} <7%, no hypos, and no weight gain, than glimepiride after 2 years of treatment regardless of duration of diabetes or age.

Success rate (SR) (HbA _{1c} < 7% , no hypos, no weight gain)				
	Vildagliptin	Glimepiride	Relative SR	CI 95%
Age (years)	n/tot (%)	n/tot (%)		
< 50	44/207 (21.3)	24/197 (12.2)	1.74	1.10 - 2.76
50-60	95/372 (25.5)	60/360 (16.7)	1.53	1.15 - 2.05
60-70	134/372 (36)	86/345 (24.9)	1.45	1.15 - 1.81
70-80	36/85 (42.4)	20/78 (25.6)	1.65	1.05 - 2.60
Duration of Diabetes (years)				
< 2	61/200 (30.5)	29/187 (15.5)	1.97	1.33 - 2.92
2-5	100/354 (28.2)	64/311 (20.6)	1.37	1.04 - 1.81
>5	148/482 (30.7)	97/482 (20.1)	1.53	1.22 - 1.91
Overall	309/1036 (29.8)	190/980 (19.4)	1.54	1.31 - 1.80

Clinical Trial Registration Number: NCT00106340

Supported by: Novartis Pharmaceuticals

PS 069 Newer agents: post-prandial glucose, triglycerides and hypoglycaemia

834

The minimal blood glucose variability is observed in type 2 diabetic patients on metformin plus DPP-IV inhibitors

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Background and aims: HbA_{1c} reflects average blood glucose (BG) during last three months before testing. No doubts about an association between HbA_{1c} value and risk of diabetic complications. There are some reasons to suspect but not enough evidence to confirm the influence of blood glucose variability (BGV) as on this risk as on safety of glucose lowering treatment. We aimed to define is there if at all a difference in BGV in type 2 diabetic patients (T2DP) with the similar HbA_{1c} level on different treatment strategies. **Materials and methods:** We studied 94 (45F/49M) T2DP with similar HbA_{1c} in first three to seven month after initiation of different additional treatment added to Metformin in doses not less than 2g/24 since Metformin only became not enough to reach the target HbA_{1c} range. The age was 54.0±5.5y (M±SD), the diabetes duration was 5.3±1.1y, and HbA_{1c} was 7.2±0.4%. 34 T2DP (Group 1) were on sulfonylureas (Gliclazide or Glimepiride), 27 (Group 2) - on DPP-IV inhibitors (Sitagliptine or Saxagliptine), and 33 (Group 3) - on basal insulin (NPH). Groups were matched for gender, age, BMI and diabetes duration. No significant difference between groups in HbA_{1c} on the beginning of an additional treatment was seen. BGV was assessed with continuous glucose monitoring (CGM) system. CGM system high limit of BG was fixed on 9,0mmol/l, low limit - on 4,0mmol/l.

Results: An average monitoring time was near to 72 hours and numbers of sensor values were not differed between groups. In the course of CGM an average BG was 8.5mmol/l, 8.5mmol/l and 8.4mmol/l; min-max BG deviations: 2.7-11.9mmol/l, 3.4-11.4mmol/l and 2.2-14.2mmol/l in Groups 1, 2 and 3 respectively. The table shows CGM results (values are presented as Mean±SD). Table 1.

Index	Groups			P		
	1	2	3	1/2	1/3	2/3
HbA _{1c} (%)	7.22±0.35	7.23±0.43	7.19±0.22	>0.5	>0.5	>0.5
Number of sensor values	873.49±14.61	869.92±18.25	871.41±19.18	>0.5	>0.5	>0.5
Duration above high limit (%)	12.32±4.18	8.50±3.36	13.94±5.80	<0.05	>0.05	<0.05
Duration below low limit (%)	22.52±7.32	8.95±5.34	26.73±8.08	<0.02	<0.05	<0.01
Duration within limits (%)	65.16±5.86	82.55±5.19	59.33±7.92	<0.01	<0.05	<0.001

Conclusion: BGV in different T2DP with equal HbA_{1c} could be significant. The minimal BG excursions were found on DPP-IV inhibitors and the most meaningful were on NPH added to Metformin. How much BGV is clinically important remains unclear and needs further study.

835

The interaction of colessevelam with sitagliptin suggests differential effects on fasting and postprandial glucose metabolism in subjects with type 2 diabetes

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Background and aims: Lipid lowering therapy with a bile-acid sequestrant such as colessevelam in people with type 2 diabetes has been associated with a small but significant decrease in HbA_{1c}. However the mechanism of action is unclear, despite suggestions that this is mediated by the incretin pathway. Dipeptidyl peptidase-4 (DPP-4) inhibitors such as sitagliptin raise concentrations of active Glucagon-Like Peptide-1 (GLP-1). As part of a study examining the mechanism of action of colessevelam on glucose metabolism, we examined the interaction of these two compounds and its effects on fasting and postprandial glucose turnover in people with type 2 diabetes.

Materials and methods: We studied 40 subjects with type 2 diabetes treated with metformin using a double blind, placebo-controlled parallel group de-

sign. Upon enrollment, subjects had a standardized, labeled mixed meal labeled with [$1\text{-}^{13}\text{C}$]-glucose. The previously-described triple-tracer technique enabled simultaneous measurement of the systemic rate of meal appearance (Meal Ra), endogenous glucose production (EGP) and glucose disappearance (Rd). Subjects were then randomized to daily treatment with 3.75g of colesevelam or placebo and after a 12-week treatment period, the mixed meal test was repeated. All subjects then participated in an 8-week extension study where they were treated with a combination of colesevelam and sitagliptin, after which the mixed meal test was repeated again. Insulin action and β -cell responsivity (ϕ_{Total}) were calculated using the oral minimal model.

Results: Colesevelam and sitagliptin had an additive effect on fasting and postprandial glucose concentrations. Unlike sitagliptin, colesevelam lowered fasting EGP while not affecting insulin secretion or action g in response to the meal challenge. Sitagliptin did not alter insulin action but stimulated insulin secretion.

Conclusion: The net effects of colesevelam alone on glucose metabolism (decreased EGP, unchanged glucagon concentrations and Disposition Indices) suggest that its effect is not mediated via the incretin system. Nevertheless, it interacts with DPP-4 inhibition to further lower glucose concentrations due to the effect of the latter compounds on insulin secretion (ϕ_{Total}). Intriguingly, colesevelam weakly increases total GLP-1 concentrations perhaps via effects on gastrointestinal motility.

	Fasting glucose (mmol/L)	Integrated Glucose (mol per h)	EGP ($\mu\text{mol/kg/min}$)	ϕ_{Total} (10^{-2}min^{-1})	Peak Total GLP-1 (pmol/L)
Baseline	7.05 \pm 0.24	3.28 \pm 0.14	17.4 \pm 0.6	24.4 \pm 1.9	37.5 \pm 5.1
Colesevelam	6.56 \pm 0.20	3.03 \pm 0.13	16.8 \pm 0.6	26.9 \pm 1.8	43.6 \pm 4.0
C + S*	6.15 \pm 0.20	2.87 \pm 0.11	16.7 \pm 0.9	34.6 \pm 1.9	45.4 \pm 5.0
P	< 0.01	< 0.01	0.04	0.03	0.01

Data are shown as mean \pm SEM, * S = sitagliptin

Clinical Trial Registration Number: NCT00951899

Supported by: Daiichi Sankyo

836

Effects of 10-month sitagliptin therapy on HbA_{1c}, triglyceride and remnant cholesterol levels in type 2 diabetic patients

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Background and aims: In 2009, sitagliptin became available in Japan. Sitagliptin is an orally active, potent and selective dipeptidyl peptidase-4 (DPP-4) inhibitor for the treatment of type 2 diabetic patients. Sitagliptin acts through increasing incretin (GLP-1 and GIP) hormone concentration, and reduces plasma glucose and HbA_{1c} levels. In addition, DPP-4 inhibitor was reported to reduce plasma triglyceride (TG) level. Remnant lipoproteins which underlie hypertriglyceridemia are known to be atherogenic as well as LDL. However, there is little information about the effect of long-term sitagliptin therapy on HbA_{1c}, TG and remnant cholesterol levels in Japan. The effects of 10-month add-on therapy with sitagliptin on HbA_{1c}, TG and remnant cholesterol levels were examined in Japanese type 2 diabetic patients. In addition, obesity and/or disease duration may impact patient therapeutic response to medication. Thus, this study also evaluated the effect of obesity and/or diabetes duration on glycemic response to sitagliptin therapy in patients whose type 2 diabetes was not optically controlled with glimepiride or pioglitazone.

Materials and methods: Fifty five patients with type 2 diabetes and baseline HbA_{1c} (JDS) $\geq 6.2\%$ to $\leq 7.5\%$ were studied. Mean age was 63 years. All patients were treated with glimepiride (mean dose 1.5 ± 0.1 mg/day) at least for 3 months before the study entry. Sitagliptin 50mg/day was added on after the dose of glimepiride (mean dose 0.6 ± 0.1 mg/day) was reduced to approximately half. Patients were treated with sitagliptin add-on glimepiride over 10 months. HbA_{1c} and plasma lipid levels were compared before and 10 months after add-on therapy with sitagliptin. HbA_{1c} was measured by high-performance liquid chromatography. Plasma remnant cholesterol was determined as RLP-cholesterol (normal range < 5.2 mg/dl) by the method of Nakajima et al. Obesity was defined as BMI ≥ 25 kg/m² according to the criteria in Japanese.

Results: Overall, 10-month add-on therapy with sitagliptin significantly reduced HbA_{1c} level ($6.8 \pm 0.1\% \rightarrow 6.1 \pm 0.1\%$, $p < 0.001$). Reduction in HbA_{1c} from baseline at 10 months was significantly ($p < 0.01$) greater in patients with obesity and diabetes duration < 10 years. Overall, 10-month add-on therapy with sitagliptin significantly reduced TG level ($146 \rightarrow 106$ mg/dl, $p < 0.001$) and

remnant cholesterol level ($6.1 \rightarrow 4.0$ mg/dl, $p < 0.001$). There was no significant change in LDL-cholesterol and HDL-cholesterol levels before and after add-on therapy with sitagliptin. Body weight was not significantly changed, and no adverse reactions such as hypoglycemia were observed over the study period.

Conclusion: It is concluded that 10-month add-on therapy with sitagliptin is effective to reduce plasma TG and remnant cholesterol levels as well as HbA_{1c} in Japanese type 2 diabetic patients with HbA_{1c} $\leq 7.5\%$. In addition, sitagliptin therapy is more effective to reduce HbA_{1c} level in patients with obesity and diabetes duration < 10 years. This is the first report that DPP-4 inhibitor reduces plasma remnant cholesterol.

837

The DPP-4 inhibitor alogliptin reduces postprandial TG and TG-rich lipoproteins in type 2 diabetes

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Background and aims: Pharmacological inhibition of DPP-4 leading to augmentation of GLP-1 receptor signalling has recently been shown to reduce intestinal lipoprotein secretion in experimental studies, suggesting that DPP-4 inhibitors may offer a novel approach of reducing cardiovascular risk in patients with T2DM. We assessed the effects of alogliptin (ALO) and alogliptin co-administered with pioglitazone (ALO/PIO) compared with placebo (PBO) on postprandial triglyceride (TG)-rich lipoproteins in a randomized clinical trial in subjects with T2DM.

Materials and methods: Double-centre, randomized, double-blind, placebo-controlled, parallel-group study of 16-week duration. Seventy-one patients (age 18–70 years) having failed treatment with diet and exercise, or a stable dose of metformin, sulfonylurea, or glinides for more than three months were recruited. Entry criteria included a baseline HbA_{1c} of 6.5–9.0%, fasting plasma glucose of < 13.3 mmol/L, fasting TG (FTG) of 1.7–5.0 mmol/L, BMI of 23–45 kg/m², an apo E3/3 or E3/4 phenotype, and a stable statin and/or ezetimibe therapy or no lipid-lowering therapy within 3 months prior to screening.

Results: At 16 weeks, ALO reduced FTG by 0.56 mmol/L and ALO/PIO lowered FTG by 0.81 mmol/L compared to PBO ($p = 0.003$ and $p < 0.001$, respectively). Treatment with ALO/PIO significantly increased fasting HDL-C (0.23 mmol/L) from baseline compared to PBO ($p < 0.001$). Both ALO and ALO/PIO produced similar and statistically significant ($P < 0.001$) reductions at week 16 in total postprandial TG response (incremental area under the curve [IAUC]) compared to PBO (TG IAUC reduced by 3.47 vs. 2.87 mmol²/h/L, respectively, NS). Furthermore, both ALO and ALO/PIO led to similar, significant reductions in chylomicron TG and VLDL1-TG IAUCs compared to PBO. Postprandial chylomicron B-48 IAUC showed a significant decrease after treatment with ALO (0.62 mg²/h/L, $p = 0.028$), and a non-significant trend towards a decrease with ALO/PIO (0.33 mg²/h/L, $p = 0.213$). Both ALO and ALO/PIO treatment produced statistically significant reductions in chylomicron cholesterol IAUC at week 16 compared to PBO ($p < 0.001$ and $p = 0.002$, respectively). HbA_{1c} at baseline averaged 6.6%, 6.8% and 6.6% in patients treated with PBO, ALO and ALO/PIO, respectively. In patients randomized to PBO HbA_{1c} increased by 0.4% after 16 weeks to 7.0%, while it decreased by 0.43% to 6.37% and by 0.93% to 5.86% in patients randomized to ALO and ALO/PIO, respectively ($P < 0.001$ for both treatment arms). Treatment with ALO/PIO decreased HbA_{1c} to a significantly greater extent than ALO alone ($P = 0.005$).

Conclusion: Treatment with ALO and ALO/PIO produced reductions in both fasting and postprandial TG and TG-rich lipoproteins as well as HbA_{1c}, contributing to an improved risk factor profile for cardiovascular disease in patients with T2DM and elevated TG levels.

Clinical Trial Registration Number: NCT00655863

Supported by: Takeda Global Research & Development.

838

Effects of sitagliptin, acarbose and sulfonylureas on postprandial levels of GLP-1 and GIP in Japanese patients with type 2 diabetes

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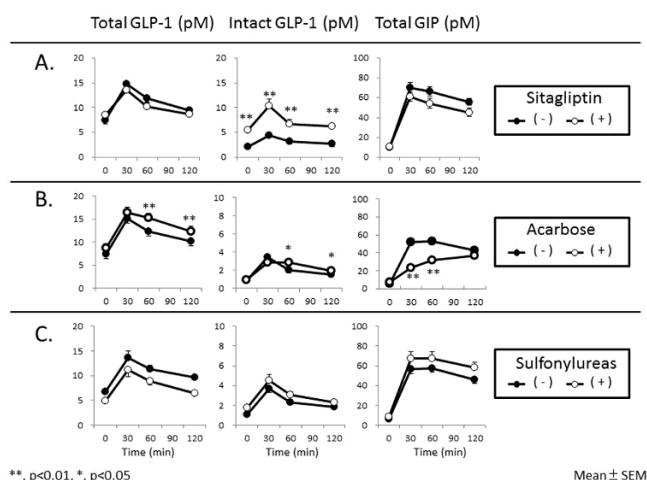
Background and aims: We previously demonstrated that meal-induced GLP-1 secretion is negligible in the Japanese. Thus, therapeutic approaches to enhance GLP-1 secretion or to prevent its degradation could be effective to improve glycemic control in Japanese patients with type 2 diabetes (T2DM). Here, we evaluated effects of sitagliptin, acarbose and sulfonylureas (SU) on postprandial levels of GLP-1 as well as GIP, glucose, insulin and glucagon levels in Japanese T2DM patients.

Materials and methods: Japanese T2DM patients were subjected to meal-tolerance tests using a Japanese standard breakfast (480 kcal; carbohydrate: protein: fat=2.8: 1: 1) with or without drug administration, and incretin levels as well as glucose, insulin, and glucagon levels were determined. To measure incretins, plasma samples were subjected to solid-phase extraction prior to immunoassays. Intact GIP levels were not measured because immunoassays were unavailable.

Results: Two-week administration of once daily 100 mg sitagliptin in 27 patients [Age (year), 62 ± 2 ; BMI (kg/m²), 25.1 ± 1.2 ; HbA_{1c} (NGSP, %), 7.4 ± 0.2] significantly elevated fasting and postprandial levels of intact GLP-1, and significantly increased the area under the curve (AUC) of intact GLP-1 levels after meal ingestions, while total GLP-1 and GIP levels did not change (Figure 1A). One time administration of 100 mg acarbose in 21 patients [Age (year), 61 ± 1 ; BMI (kg/m²), 23.2 ± 0.5 ; HbA_{1c} (NGSP, %), 7.0 ± 0.1] slightly but significantly increased both total and intact GLP-1 levels 60 and 120 min after meal ingestions as well as total GLP-1 AUC (Figure 1B). The acarbose administration significantly decreased total GIP levels 30 and 60 min after meal ingestions as well as total GIP AUC. Postprandial incretin levels in 23 untreated and 18 SU-treated patients [Age (year), untreated 59 ± 2 and SU 67 ± 1 ; BMI (kg/m²), untreated 22.0 ± 0.5 and SU 23.0 ± 0.8 ; HbA_{1c} (NGSP, %), untreated 7.0 ± 0.1 and SU 7.3 ± 0.1] did not differ between two groups (Figure 1C).

Conclusion: In Japanese T2DM patients, sitagliptin, through inhibition of DPP-4-dependent degradation, increased intact GLP-1 levels without affecting secretion of GLP-1 and GIP. Acarbose enhanced GLP-1 secretion and suppressed GIP secretion after meal ingestions. Sulfonylureas had little effects on meal-induced secretion of GLP-1 and GIP.

Figure 1



Supported by: the Japan Diabetes Foundation and the Diabetes Masters Conference

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Better adherence with vildagliptin add-on to metformin than sulphonylurea add-on to metformin among Muslim patients with type 2 diabetes mellitus fasting during Ramadan

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Background and aims: During the holy month of Ramadan, Muslim patients with type 2 diabetes mellitus (T2DM) who fast are at increased risk of hypoglycaemia and poor glycaemic control. Factors that can reduce these risks include choice of oral antidiabetes drug (OAD) therapy and patient adherence to treatment. In this study, patients receiving one of two different combination OAD therapies were monitored before, during and after their Ramadan fast. One objective of the study was to assess the number of OAD doses missed during the fasting period, according to therapy.

Materials and methods: This prospective, observational cohort study was conducted in four UK centres. Patients already taking vildagliptin or a sulphonylurea (SU) as add-on therapy to metformin were followed for ≤ 16 weeks (maximum of 6 weeks before and 6 weeks after the fasting period) and asked to record how many doses they missed. Glycated haemoglobin (HbA_{1c}) and hypoglycaemic events (HEs), defined as any symptoms reported by the patient or any blood glucose measurements < 3.9 mmol/l, were also recorded.

Results: Of the 72 patients enrolled (vildagliptin, $n = 30$; SU, $n = 41$; not allocated to treatment, $n = 1$), 59 (81.9%) completed the study (vildagliptin, $n = 23$; SU, $n = 36$) including one patient in the SU arm who completed but failed to provide information on missed doses; all patients in the SU arm were taking gliclazide. In the vildagliptin arm, one patient (4.3%) missed a total of four doses; in the SU arm, ten patients (27.8%) missed a total of 266 doses (mean [SD] number of doses missed per patient: 26.6 [16.5]). The mean (SD) proportion of doses missed during fasting was 0.2% (0.9%) in the vildagliptin arm and 10.4% (21.7%) in the SU arm, with a significant mean between-group difference of -10.2% (95% CI: -19.3% , -1.1%), $p = 0.0292$. No patients reported HEs in the vildagliptin arm and 15 patients (42%) reported 34 HEs (including one grade 2 HE) in the SU arm. Of these 15 patients, more were adherent ($n = 9$, 60%) than were not ($n = 6$, 40%). At baseline, HbA_{1c} was 7.7% in the vildagliptin arm and 7.2% in the SU arm. After fasting, the mean between-group difference (vildagliptin minus SU) was -0.5% (95% CI: -0.9% , -0.1%), $p = 0.0262$.

Conclusion: During Ramadan fasting, almost all patients with T2DM receiving vildagliptin adhered to treatment and none reported HEs. In contrast, nearly half of patients receiving an SU experienced HEs and almost one-third missed doses. Of note, the majority of patients who experienced HEs were adherent. Our findings highlight the importance of choosing an OAD therapy that matches a patient's lifestyle, and suggest that vildagliptin is a suitable treatment option in Muslim patients who fast.

Supported by: Novartis Pharmaceuticals UK

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Real life experience of usage of vildagliptin versus sulphonylurea therapy in fasting patients with type 2 diabetes during Ramadan: an Indian experience

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Background: Majority of Indian Muslim patients observe the fast during holy month of Ramadan. There is limited data available for Vildagliptin, a selective and orally-effective inhibitor of DPP-IV, which increases active (intact) GLP-1 levels, lowers meal-stimulated glucagon levels, improves glucose tolerance and improves beta cell function; in type 2 diabetes (T2DM) patients. The purpose of this trial was to assess efficacy and safety of Vildagliptin in real day-to-day clinical practice in Indian T2DM patients fasting during Ramadan.

Objective: To compare hypoglycemic events, changes in body weight and change in HbA_{1c}, Fasting blood sugar (FBS) & Postprandial blood sugar (PPBS) levels in Indian Muslim T2DM patients, treated with Vildagliptin or Vildagliptin+metformin combination Vs. sulphonylurea or sulphonylurea+metformin combination, during Ramadan fast.

Materials and methods: A total of 97 patients with mean age of 50.95 years were recruited in this open label, observational, prospective, comparative

study, across 10 centers in India. Patients were randomly selected to receive Vildagliptin or Vildagliptin+metformin (N=55) or sulphonylurea or sulphonylurea+metformin combination (N=42), and observed for the fasting period of 4 weeks (29 days).

Results: The reduction in HbA1c levels pre & post Ramadan (time frame of 4 weeks) was significant at -0.43% (8.75% vs. 8.32%; $p=0.009$; 95%CI) in Vildagliptin group; in contrast there was slight increase in levels of HbA1c by 0.01% (8.64% vs. 8.65%; $p=0.958$; 95%CI) in sulphonylurea group. Also 9(16.37%) more patients reached target HbA1c goal <7% as compared to only 2(4.76%) patients in sulphonylurea group ($p=.055$). During Ramadan, 2(4.76%) hypoglycemic events (1 with glipizide 5mg BID and other with glibenclamide 5mg BID) were reported in the sulphonylurea group (baseline HbA1c=8.64%) compared to none in vildagliptin group (baseline HbA1c=8.75%) ($p=0.104$). The mean FBS & PPBS levels decreased significantly in both the group. Vildagliptin was also associated with a mean weight reduction of -1.02kg (69.13kg vs. 67.93kg; $p=0.000$; 95%CI) pre & post Ramadan, whereas sulphonylurea group had negligible mean weight reduction during fasting period. The between the group difference for mean weight reduction was again statistically significant (-1.02Kg Vs. -0.03Kg; $p=0.011$; 95%CI). The between the group difference for reduction in mean FBS & PPBS levels were not statistically significant.

Conclusion: Like other T2DM patients, improved glycemic control without hypoglycemia is essential in fasting diabetic patients. Unlike sulphonylurea therapy, vildagliptin based therapy offers improved glycemic control with significant HbA1c reduction and better safety with weight reduction and no hypoglycemia event in fasting T2DM Indian patients.

Supported by: Novartis India Limited

PS 070 SGLT inhibitors: new agents and clinical considerations

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Characterisation of urinary tract infections in the setting of pharmacologically induced glucosuria

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Background and aims: Type 2 diabetes mellitus (T2DM) predisposes patients to urinary tract infection (UTI). While glucose added to urine has been reported to increase *E. coli* growth in vitro, epidemiological data have not consistently shown an association between increased urinary excretion and UTI. Pooled data from controlled clinical trials with dapagliflozin, an SGLT2 inhibitor that improves glycaemia in patients with T2DM via a dose-dependent increase in renal glucose excretion, offer an opportunity to test this hypothesis.

Materials and methods: The data described here were pooled from 12 randomized, placebo-controlled studies ranging from 12 to 24 weeks in treatment duration. Patients were actively questioned at each trial visit to identify possible urinary tract infections, and the results were evaluated using two analysis methods. In a broad analysis for safety signal detection, the presence of signs and symptoms suggestive of urinary tract infection (e.g., dysuria) as well as clinical diagnoses of urinary tract infection were assessed based on a citation of a set of 63 prespecified MedDRA-preferred terms (PTs). For more specific safety signal quantification the analysis was limited to 49 PTs describing clinically diagnosed infections.

Results: The incidence of signs, symptoms, and events suggestive of UTI with dapagliflozin 2.5 mg was similar to placebo and was higher with dapagliflozin 5 and 10 mg, suggesting a relevant safety signal (Table). Diagnoses of infection were also higher for dapagliflozin 5 and 10 mg than placebo, allowing more specific quantification of this safety signal. Actual events collected during interviews mapped to a limited subset of the PTs. Most signs, symptoms, and events suggestive of UTIs did not recur in the 12-24 weeks, and patients with a history of recurrent UTI were more likely to have an event during the study than those without a prior history. Urine cultures were obtained in ~1/2 of the suggestive events and ~2/3 of these cultures were positive. The majority of events were mild to moderate, and few patients discontinued or interrupted treatment as a consequence. UTIs in all of the dapagliflozin groups were qualitatively similar to those in the placebo group and were also similar to the general T2DM population with respect to pathogens, complications and response to antibiotics. Events of pyelonephritis were infrequent in all groups (Table).

Conclusion: These results suggest that increased renal glucose excretion with the SGLT2 inhibitor dapagliflozin (at doses ≥ 5 mg/day) is accompanied by an elevated incidence of signs, symptoms, and events suggestive of UTI. The actual rates are dependent on the specificity of the PTs analyzed.

Table

		Placebo	Dapagliflozin 2.5 mg/d	Dapagliflozin 5 mg/d	Dapagliflozin 10 mg/d
	N	1393	814	1145	1193
Events Suggestive of UTI (%)	Total	4.5	4.2	7.3	6.5
Clinically	Total	3.7	3.6	5.7	4.3
Diagnosed UTI (%)	Women	6.6	5.8	9.6	7.7
	Men	1.0	1.4	1.6	0.8
Pyelonephritis (n)	Total	1	2	1	0

Supported by: AZ and BMS

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Dapagliflozin selectively inhibits human SGLT2 versus human SGLT1, SMIT, SGLT4, SGLT6, GLUT1, GLUT2 and GLUT4

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Background and aims: Dapagliflozin, an SGLT2 inhibitor that reduces renal glucose reabsorption and may potentially provide an insulin-independent therapy for the treatment of Type 2 diabetes, is 3,000-fold selective for human SGLT2 vs. human SGLT1 based on K_i values. Here we report selectivity versus other glucose transporters.

Materials and methods: SMIT (SLC5A3), SGLT4 (SLC5A9) and SGLT6 (SLC5A11) were stably expressed in CHO cells. SGLT 1, 2 and 4 activity was assessed with sodium-dependent ¹⁴C- α -methyl glucopyranoside (AMG) uptake. SMIT and SGLT6 activity was assessed with sodium-dependent ¹⁴C-myoinositol uptake. The uptake was carried out at 37°C for 2 hr in presence of phlorizin at 50–500 μ M or dapagliflozin at 0.5–500 μ M. Activity of dapagliflozin at human GLUT1, 2 and 4 transporters was assessed in human erythrocytes, Hep G2 cells and human differentiated adipocytes, respectively. GLUT1 activity was measured using D-[6-³H] glucose transport. GLUT2 activity was assessed using 2-deoxy-D-[1-³H] glucose transport. GLUT4 activity was measured using insulin stimulated 2-deoxy-[U-¹⁴C]-glucose. Dapagliflozin was pre-incubated for an hour prior to the administration of the relevant glucose analogue over the concentration range 20–100 μ M to assess GLUT selectivity

Results: The K_i values for the inhibition by dapagliflozin at the SGLT isoforms and the inhibition of GLUT isoforms are described in the table below. We have demonstrated that dapagliflozin is highly selective for human SGLT2 and the inhibition constants for dapagliflozin vs. SGLT1, SMIT, SGLT4 and SGLT6 are 3000, 70,000, 16,500 and 4,050-fold higher than for SGLT2. Phlorizin is 250-fold less potent than dapagliflozin at SGLT2, and is 3-fold, 3,000-fold, 110-fold and 260-fold selective vs. SGLT1, SMIT, SGLT4 and SGLT6, respectively. Dapagliflozin had no effect on the insulin EC_{50} value for glucose uptake in the human adipocytes. Dapagliflozin also had minimal effect at GLUT1 and GLUT2 at 100 μ M. Although dapagliflozin inhibited GLUT4 by 23±4% at 100 μ M, a concentration of 20 μ M had minimal effect on the maximum response (8 ± 2% reduction). Positive response to cytochalasin B and/or phloretin were observed for all systems studied in the GLUT transport studies. This data also shows that the compound has at least 100,000-fold selectivity over GLUT1, 2 and 4.

Conclusion: At concentrations achieved following administration of therapeutic concentrations of dapagliflozin, we do not expect off-target effects attributable to inhibition of SGLT1, SGLT4, SMIT or SGLT6 transporters or GLUT1, 2 or 4 proteins or adverse effects upon insulin stimulated glucose disposal.

Table

SGLT Isoform	SGLT1	SGLT2	SGLT3	SGLT4	SGLT6
System Name	SLC5A1	SLC5A2	SLC5A3	SLC5A9	SLC5A11
Dapagliflozin/ K_i (μ M)	0.61 ± 0.18	2.10 × 10 ⁻⁴ ± 6.10 × 10 ⁻⁵	14 ± 2	3.3 ± 0.7	0.81 ± 0.12
Glut Isoform	GLUT1	GLUT2	GLUT4		
Inhibition (%) (with 100 μ M dapagliflozin)	3.6 ± 3.6	11.6 ± 3.2	23 ± 4		
Inhibition (%) (with 20 μ M dapagliflozin)	1.6 ± 3.6	3.3 ± 4.2	8 ± 2		

Supported by: AZ and BMS

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Dapagliflozin, a selective SGLT2 inhibitor, reduces serum levels of uric acid in patients with type 2 diabetes

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Background and aims: Serum uric acid is an emerging marker for cardiovascular and renal disease risk. Dapagliflozin, a selective inhibitor of the renal sodium-glucose cotransporter 2 (SGLT2), reduces hyperglycaemia in patients with type 2 diabetes independently of insulin action or secretion by increasing urinary glucose excretion, and has been associated with reductions in serum uric acid. The effect of dapagliflozin on changes in uric acid from data collected in five Phase 3 studies is reported; the main findings of these studies have been previously reported.

Materials and methods: Comparative changes from baseline in serum uric acid are reported for a 24-week study of dapagliflozin monotherapy in treatment-naïve patients inadequately controlled with diet and exercise (NCT00528372); a 102-week study of dapagliflozin as add-on to metformin in patients inadequately controlled by metformin alone (NCT00528879); a 52-week study of dapagliflozin versus glipizide (both titrated to goal) as add-on to metformin in patients inadequately controlled by metformin alone (NCT00660907); a 48-week study of dapagliflozin as add-on to glimepiride in patients inadequately controlled by glimepiride alone (NCT00680745); and a 48-week study of dapagliflozin as an add-on to insulin in patients inadequately controlled by insulin ± other oral antidiabetic drugs (NCT00673231). Serum uric acid was recorded as either an exploratory efficacy variable (NCT00528372, NCT00528879) or as a safety variable (NCT00660907, NCT00680745, NCT00673231). Urinary uric acid levels were not examined in these studies.

Results: Mean serum uric acid reductions from baseline with dapagliflozin treatment ranged from 11.3 to 59.5 μ mol/L, and were sustained for up to 102 weeks. Placebo and glipizide treatment were not associated with uric acid reductions.

Changes in Serum Uric Acid in Dapagliflozin Studies

Study ID	Design	Dose of dapagliflozin or comparator (mg/day)	N	Time from baseline (weeks)	Mean baseline serum uric acid (μ mol/L)	Mean change from baseline (μ mol/L)	Confidence/error (95%CI) or [SE]
NCT00528372	Monotherapy*	2.5 QAM	65	24	352.2	-39.3†	(-51.2, -27.4)
MB102-013		5 QAM	64		330.2	-50.6†	(-62.5, -38.7)
		10 QAM	70		337.4	-51.8†	(-63.1, -40.5)
		2.5 QPM	67		320.1	-59.5†	(-70.8, -47.6)
		5 QPM	68		303.5	-43.4†	(-54.7, -31.5)
		10 QPM	76		326.7	-49.4†	(-60.7, -38.1)
		Placebo	75		303.5	-11.9†	(-22.6, -0.6)
NCT00528879	Add-on to metformin*	2.5	137	102	321.7	-55.9	(-73.2, -38.6)
MB102-014		5	137		315.7	-46.6	(-65.5, -27.7)
		10	135		321.6	-53.1	(-70.2, -36.1)
		Placebo	137		310.5	-1.9	(-22.9, 19.1)
NCT00660907	Dapagliflozin versus glipizide	Dapagliflozin up to 10§	406	52	336.1	-45.2	[3.4]
D1690C00004	add-on to metformin (titration to goal)‡	Glipizide up to 20§	408		323.6	16.1	[3.4]
NCT00680745	Add-on to glimepiride‡	2.5	154	48	301.6	-17.2	[5.4]
		5	145		303.9	-17.8	[5.5]
		10	151		301.0	-26.2	[4.7]
D1690C00005		Placebo	146		315.2	20.2	[5.5]
NCT00673231	Add-on to insulin‡	2.5	202	48	326.0	-11.3	[4.7]
		5	212		323.6	-12.5	[4.7]
		10	196		324.8	-14.3	[4.9]
		Placebo	197		333.7	4.2	[4.8]

*Exploratory efficacy variable; †Data are adjusted means from ANCOVA (last observation carried forward); ‡Data from safety analysis sets; §mean titrated dose 16.4 mg for glipizide and 9.2 mg for dapagliflozin; N = number of patients randomized and treated; QAM = morning administration; QPM = evening administration.

Conclusion: Dapagliflozin consistently lowered serum uric acid levels across five Phase 3 studies examining a range of different clinical scenarios. Whether this is a direct effect on a uric acid transporter or an indirect effect related to urinary glucose excretion is unknown. Further research is needed to establish the clinical significance of modest uric acid reductions in patients with type 2 diabetes who have normal baseline uric acid levels.

Supported by: AstraZeneca and Bristol-Myers Squibb

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The sodium glucose co-transporter-2 (SGLT2) inhibitor, PF04971729, yielded BP lowering in hypertensive patients with type 2 diabetes mellitus

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Background and aims: PF04971729 is a highly potent and selective SGLT2 inhibitor in development for treatment of T2DM. This phase 2, randomized, placebo-controlled, double-blind, double-dummy study investigated the efficacy and safety of PF04971729 in patients with hypertension (HTN) and T2DM. At screening, patients were on 1 or 2 oral anti-diabetic agents (excluding thiazolidinediones), plus up to 2 anti-hypertensive agents with those affecting renin-angiotensin-aldosterone-system (RAAS) stopped 3 weeks prior to randomization.

Materials and methods: A total of 194 patients (32% female) were randomized, to once daily oral doses of placebo, 1 of 3 doses of PF04971729, or 12.5 mg hydrochlorothiazide (HCTZ), for 4 weeks. Mean baseline characteristics were HTN duration 6 years, T2DM duration 7 years, HbA1c 8.2%, 24-h average BP 136/81 mmHg (ambulatory blood pressure monitoring - ABPM) and 136/84 mmHg (seated, trough measurements). Efficacy endpoints were 24-h, day-time, and night-time BP, as well as seated, trough BP, markers of RAAS, and fasting plasma glucose (FPG). Assessments of safety and tolerability were also included. For endpoints with 1 post-treatment measure at Week 4, analysis of covariance (ANCOVA) was applied on observed data; for endpoints with more than 1 measurement post-randomization, mixed model repeated measures (MMRM) was utilized on all observed data.

Results: A total of 184 subjects completed the study. The table below summarizes the results. The frequency of adverse events (AEs) was similar across all arms with none of the subjects withdrawn due to AEs. There were no cases of pyelonephritis reported. Urinary tract infections were reported in a total of 5 patients (1 on placebo, 4 on PF04971729); genital fungal infections were reported in 4 patients (all on PF04971729).

Conclusion: In patients with HTN and T2DM, administration of once-daily PF04971729 for 4 weeks resulted in a clinically meaningful decrease in BP along with improvement in glycemic control. The BP lowering efficacy, at least comparable to that of HCTZ, warrants further investigation in combination with ACE-inhibitors or angiotensin receptor blockers.

Change from Baseline at Week 4 in BP, Markers of RAAS, FPG

	Placebo	PF04971729 1mg	PF04971729 5mg	PF04971729 25mg	HCTZ 25mg
Subjects Randomized	39	39	38	39	39
24-h SBP (DBP) - mmHg*	0.2 ± 1.17 (0.7 ± 0.78)	-2.7 ± 1.10 ^a (-1.9 ± 0.73 ^a)	-3.7 ± 1.21 ^a (-2.4 ± 0.81 ^a)	-3.4 ± 1.12 ^a (-1.5 ± 0.74 ^a)	-3.1 ± 1.13 ^a (-1.4 ± 0.75 ^a)
Day-time SBP (DBP) - mmHg*	0.8 ± 1.26 (0.8 ± 0.82)	-3.0 ± 1.18 ^a (-2.2 ± 0.76 ^a)	-3.6 ± 1.31 ^a (-1.9 ± 0.84 ^a)	-4.2 ± 1.20 ^a (-1.8 ± 0.78 ^a)	-3.2 ± 1.22 ^a (-1.6 ± 0.78 ^a)
Night-time SBP (DBP) - mmHg*	-0.3 ± 1.56 (1.0 ± 1.10)	-2.4 ± 1.45 (-1.5 ± 1.02 ^a)	-3.4 ± 1.61 (-2.5 ± 1.14 ^a)	-2.3 ± 1.48 (-0.9 ± 1.05)	-2.4 ± 1.50 (-0.6 ± 1.10)
Seated, trough SBP (DBP) - mmHg*	1.2 ± 1.69 (0.3 ± 0.99)	-2.8 ± 1.69 ^a (-0.9 ± 1.0)	-5.9 ± 1.71 ^a (-0.8 ± 1.0)	-5.0 ± 1.71 ^a (-2.7 ± 1.0 ^a)	-3.1 ± 1.66 ^a (-2.5 ± 0.97 ^a)
24-h Urine Volume - mL*	-26 ± 102	156 ± 103	228 ± 105 ^a	187 ± 103	-53 ± 101
24-h Urine Aldosterone - mg*	0.15 ± 1.03	0.35 ± 1.03	0.31 ± 1.06	0.19 ± 1.04	-0.41 ± 1.01
Plasma renin - ng Ang I/mL/h ^Δ	0.41 ± 0.28	-0.27 ± 0.29	0.46 ± 0.29	0.46 ± 0.29	0.27 ± 0.27
FPG - mg/dL ^Δ	4.4 ± 5.09	-13.7 ± 5.17 ^a	-30.4 ± 5.21 ^a	-31.0 ± 5.21 ^a	3.8 ± 5.07

*Least-square-mean (LSMean) ± SE using ANCOVA; ^ΔLSMean ± SE using MMRM; ^avalue (change from baseline) statistically significantly different (1-sided p-value < 0.05) compared to placebo

Clinical Trial Registration Number: NCT01096667

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Long-term treatment of TS-071, a novel, potent and selective SGLT2 inhibitor, improves hyperglycaemia and prevents the loss of beta cell in diabetic mice

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Background and aims: We have discovered a novel SGLT2 inhibitor, TS-071 (TS), which increases urinary glucose excretion and provides insulin-independent reduction of hyperglycemia in vivo. The aim of this study is to assess the in vitro profile of TS and to evaluate the long-term efficacy of this compound on hyperglycemia and β -cell injury in diabetic mice. Sitagliptin (SITA) and pioglitazone (PIO) were used as reference compounds.

Materials and methods: The in vitro potency and property of TS were evaluated using CHO-K1 cells overexpressing human SGLT2. In the in vivo experiments, high fat-fed streptozotocin-treated (HFD/STZ) mice were given orally vehicle (VEH), TS or SITA for 12 weeks. Plasma glucose (PG) and glycated hemoglobin (GHb) were measured, and the pancreas was isolated to analysis of insulin content. Eleven-week-old db/db mice were given orally VEH, TS or PIO for 8 weeks, PG, GHb and plasma triglyceride (TG) were measured. Pancreatic β -cell area was evaluated by histological analysis. To further elucidate the combination effects of TS with metformin (MET), 11-week-old db/db mice were orally given VEH, TS, MET or combined TS and MET (TS+MET) for 10 weeks. GHb and pancreatic β -cell area were assessed at the end of the study.

Results: In CHO-K1 cells overexpressing human SGLT2, TS competitively inhibited Na⁺-dependent ¹⁴C- α -methylglucoside uptake with a Ki value of 1.10 nM. In HFD/STZ mice, both TS and SITA significantly decreased the change in GHb [Δ GHb (%): VEH = 0.33 ± 0.4, TS 1 mg/kg = -0.12 ± 0.3, TS 3 mg/kg = -0.61 ± 0.3, TS 10 mg/kg = -1.09 ± 0.1*, SITA 200 mg/kg = -1.09 ± 0.3*, *p < 0.05 vs. VEH] and increased pancreatic insulin contents (μ g/g pancreas: VEH = 29.3 ± 4.9, TS 1 mg/kg = 38.8 ± 3.8, TS 3 mg/kg = 43.0 ± 4.6, TS 10 mg/kg = 53.5 ± 5.7**, SITA 200 mg/kg = 55.1 ± 10.3*, *p < 0.05, **p < 0.01 vs. VEH). In db/db mice, TS (3 mg/kg) significantly decreased PG, GHb and TG [fasting PG (mg/dL): VEH 796 ± 36 vs. TS 535 ± 22, P < 0.001; GHb (%): VEH 9.5 ± 0.3 vs. TS 6.9 ± 0.2, p < 0.001; fasting plasma TG (mg/dL): VEH 87 ± 14 vs. TS 47 ± 5, p < 0.05]. TS significantly restored the pancreas β -cell mass [insulin-positive area to total islet area (%): VEH 21.5 ± 1.6 vs. TS 31.8 ± 2.3, p < 0.01] and reduced the pancreas α -cell mass [glucagon-positive area to total islet area (%): VEH 12.1 ± 1.2 vs. TS 6.9 ± 0.7, p < 0.01]. PIO (10 mg/kg) also improved glycemic control and restored the pancreas β -cell mass, but had no effect on the pancreas α -cell mass. Combination treatment with TS (3 mg/kg) and MET (300 mg/kg) significantly reduced GHb compared with MET alone [GHb (%): MET 9.9 ± 0.4 vs. TS+MET 8.2 ± 0.4, p < 0.05]. TS+MET also demonstrated significant improvement in the pancreas β -cell mass compared with MET alone [insulin-positive area to total islet area (%): MET 13.1 ± 1.5 vs. TS+MET 23.9 ± 2.5, p < 0.01].

Conclusion: These results indicate that TS, either as monotherapy or in combination with MET, improved glycemic control and preserved β -cell mass in diabetic mice.

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TS-071, a novel potent and highly selective renal sodium-glucose co-transporter 2 (SGLT2) inhibitor, increases urinary glucose excretion and reduces plasma glucose levels in Japanese patients with type 2 diabetes mellitus

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Background and aims: Renal sodium-glucose co-transporter 2 (SGLT2) inhibition is known as a new approach for the treatment of type 2 diabetes mellitus (T2DM). TS-071 is a novel, orally bioavailable, and highly selective SGLT2 inhibitor. This is the clinical pharmacology study to evaluate pharmacodynamic (urinary glucose excretion (UGE), plasma glucose levels), pharmacokinetic (PK) and safety in Japanese patients with T2DM.

Materials and methods: In single-blind, placebo (PBO) -controlled, parallel group, clinical pharmacology study, subjects (N=40) were randomized to TS-071 0.5, 1, 2.5, 5 mg once daily (QD) or PBO for 7 days administration. Mean baseline characteristics in each group were HbA1c 7.99 - 8.70 %, fasting

plasma glucose (FPG) 150.1–166.5 mg/dL, age 55.9–59.8 years, BMI 23.4–26.8 kg/m² and body weight 66.8–76.0 kg.

Results: UGE up to 24 hours (UGE-24hr) after administration on Day 1 and Day 7 significantly increased in all the TS-071 treatment groups compared to PBO group. UGE-24hr on Day 7 was dose-dependent, and least squares means of difference between each treatment group and PBO group were 49.2, 66.5, 89.4 and 101 g in 0.5 mg, 1 mg, 2.5 mg and 5 mg group respectively. The values of plasma glucose AUC on Day 7 after breakfast and after lunch were significantly lower in all TS-071 groups compared to placebo group, and the values after supper were also significantly lower in 1 mg–5 mg groups excluding 0.5 mg group compared to PBO group. TS-071 showed reductions in plasma glucose levels throughout the day by QD administration. Plasma TS-071 concentrations increased dose-dependently. Dose-proportionality was observed in C_{max} and AUC_{0–24} on Day 7. It was assumed that there would be no effect of repeated administration such as accumulation. Plasma pharmacokinetics in T2DM patients in the 5 mg group was similar to that in healthy subjects. No major or serious safety concern was observed in all TS-071 groups. Nine adverse events occurred in 7 subjects. All the adverse events were mild in severity and recoverable. Out of these adverse events, only an adverse drug reaction of constipation was observed in a subject (0.5 mg group). There were no findings suggesting hypoglycemia and urinary tract infection. It was concluded that there would be no problem with tolerability in this study.

Conclusion: In patients with T2DM, once daily administration of TS-071 demonstrated dose-dependent UGE increase, reductions in plasma glucose levels throughout the day and showed favorable profile of PK and safety.

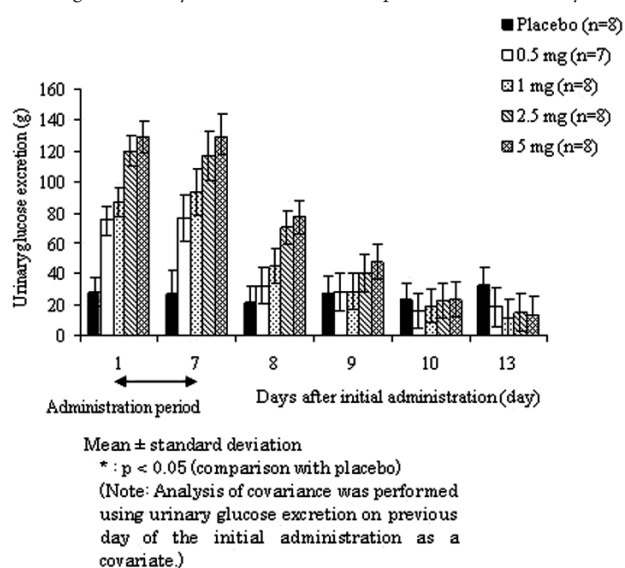


Figure 1 Changes in 24-Hour Cumulative Urinary Glucose Excretion

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The effect of renal impairment on the pharmacokinetics and urinary glucose excretion of the SGLT2 inhibitor ipragliflozin (ASP1941) in Japanese type 2 diabetes mellitus patients

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Background and aims: Sodium-dependent glucose co-transporter 2 (SGLT2) is primarily responsible for the reabsorption of glucose in the renal proximal tubules. Ipragliflozin (ASP1941) is a novel, potent, and selective SGLT2 inhibitor that induces an increase in urinary glucose excretion (UGE), thereby reducing plasma glucose levels. This study investigated the effect of different degrees of renal impairment (RI) on the pharmacokinetics of and the UGE induced by ipragliflozin, in Japanese type 2 diabetes mellitus (T2DM) patients.

Materials and methods: T2DM patients with normal renal function (NRF; estimated glomerular filtration rate [eGFR] ≥ 90 mL/min/1.73m²), and mild (eGFR 60–89 mL/min/1.73m²) and moderate (eGFR 30–59 mL/min/1.73m²) RI were enrolled. Patients were administered a single oral dose of 50 mg ip-

ragliflozin (n = 8–9 per renal function group). Plasma concentrations of ipragliflozin, UGE, and safety were assessed up to 72 hours after dosing.

Results: In T2DM patients with mild RI, the maximum plasma concentration (C_{max}) of ipragliflozin was increased by 12%, and the area under the plasma concentration-time curve from time of dosing to infinity (AUC_{inf}) was comparable to patients with NRF. In T2DM patients with moderate RI, C_{max} and AUC_{inf} of ipragliflozin were increased by 17% and 21%, respectively, compared with T2DM patients with NRF. Mean daily UGE increased in all groups. Mean changes from baseline in UGE were comparable between patients with mild RI and NRF, and lower in patients with moderate RI versus NRF patients. Mean changes from baseline in plasma glucose levels 24 hours after administration (before breakfast) were –24.9 mg/dL in T2DM patients with NRF, –11.9 mg/dL in patients with mild RI, and –4.1 mg/dL in patients with moderate RI. As for safety, a single dose of 50 mg ipragliflozin was considered safe and well tolerated in T2DM patients with normal, mildly impaired, and moderately impaired renal function.

Conclusion: Ipragliflozin exposure increased in T2DM patients with moderate RI by 21%. The UGE after ipragliflozin dosing was reduced in T2DM patients with moderate RI. Ipragliflozin was well tolerated in the study population.

Clinical Trial Registration Number: NCT01097681

848

Effects of dapagliflozin on patient reported treatment satisfaction in patients with type 2 diabetes mellitus: results from two double-blind trials

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Background and aims: Sodium-glucose co-transporter 2 (SGLT2) inhibitors represent a new treatment modality for type 2 diabetes mellitus (T2DM) which lower blood glucose by increasing urinary glucose excretion. Dapagliflozin (DAPA) is a first in class SGLT2 inhibitor in clinical development and has been shown to improve HbA1c and reduce weight, and is safe and well tolerated. However, its novel glucosuric mechanism of action warranted an evaluation of patient treatment satisfaction.

Materials and methods: Patient-reported treatment satisfaction was measured utilizing the Diabetes Treatment Satisfaction Questionnaires for status (DTSQs max score 36) and change (DTSQc max score 18). Data were collected from two randomized, double-blind, multicenter, parallel group studies (N=1411), Study 4 and Study 5, in the DAPA phase III program. Study 4 was a 2-arm, 52 week study of DAPA up to 10 mg vs glipizide (GLIP) up to 20 mg, both added to metformin (N=814), and Study 5 was a 24 week study + 24 week extension of DAPA 2.5, 5 or 10 mg vs placebo (PBO), both added to glimepiride (N=597).

Results: The overall DTSQs scores at baseline were high in all groups in both studies (>30 in Study 4, and > 27 in Study 5). In Study 4, the DTSQs showed a slight increase in both treatment groups at week 26. The DTSQc score at week 52 in Study 4 showed a higher mean value in the DAPA group (14.3 vs GLIP 13.6). In Study 5 at week 24, the DTSQs showed a slight increase in all groups and the DTSQc ranged from 13.2 to 13.6 in the DAPA groups vs 13.0 for PBO. At week 48, the DTSQs score improved further in all DAPA groups (mean change 3.5, 4.6, 4.4) vs PBO (2.2). In the single item measuring perceived frequency of hyperglycemia there was a noticeable reduction with DAPA compared with PBO at weeks 24 and 48.

Conclusion: The high level of treatment satisfaction maintained by patients in these 2 studies with ≥ 48 weeks of treatment suggests that dapagliflozin with its novel mechanism of action through glucosuria provides a well tolerated therapeutic option for patients with T2DM.

Clinical Trial Registration Number: D1690C00004/05

PS 071 SGLT inhibitors: clinical trials

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Combination treatment with ipragliflozin (ASP1941) and metformin in type 2 diabetes patients: a safety, pharmacokinetic and pharmacodynamic interaction study

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Background and aims: Ipragliflozin (ASP1941), a novel selective inhibitor of sodium-dependent glucose co-transporter 2 (SGLT2), is in clinical development for the treatment of type 2 diabetes mellitus (T2DM). Inhibition of SGLT2 by ipragliflozin reduces glucose reabsorption from the proximal tubules of the kidney, thereby enhancing urinary glucose excretion (UGE). Metformin is commonly used as first line treatment in T2DM; however, many T2DM patients do not achieve or retain glycemic targets with metformin alone, partly due to side-effects, and therefore require additional pharmacotherapy. Here we report the safety of combination treatment with ipragliflozin and metformin, the effect of ipragliflozin on the pharmacokinetics (PK) of metformin, and the effect of concomitant use of ipragliflozin and metformin on the pharmacodynamics (PD) in T2DM patients.

Materials and methods: This was a double-blind, randomized, placebo-controlled, parallel design study. T2DM patients who were stable on metformin therapy (850 mg twice-daily [bid], 1000 mg bid, or 1500 mg bid) received either ipragliflozin 300 mg or placebo once daily (qd) for 14 days. Safety outcomes, comprising adverse events (AEs), laboratory measurements and vital signs, were assessed during the study. PK of metformin was determined in plasma using a validated liquid chromatography-tandem mass spectrometry method. PD was assessed by measurement of UGE.

Results: In total, 36 T2DM patients were included in the study ($n = 18$ per group), and received either ipragliflozin (mean age \pm standard deviation [SD] = 59 ± 8.5 years) or placebo (mean age \pm SD = 56 ± 10.9 years). The most frequent treatment emergent AEs were gastrointestinal (GI) disorders (a known and common AE of metformin), and these were reported both in ipragliflozin and placebo-treated patients (3 out of 18 patients in each group). GI disorders in the ipragliflozin group comprised diarrhea (mild, $n = 1$), dry mouth (mild, $n = 1$), nausea (moderate, $n = 1$), and vomiting (moderate, $n = 1$). Diarrhea (mild, $n = 1$) and flatulence (mild, $n = 2$) were reported in the placebo group. No symptomatic or asymptomatic (finger prick < 3 mmol/L) hypoglycemic events were reported. The change from baseline in plasma exposure (area under the concentration-time curve [AUC]) of metformin on Day 14 was slightly higher in the ipragliflozin group (AUC_{0-10h} ratio Day 14/baseline = 1.18; 90% confidence interval [CI] 1.08–1.28) than in the placebo group (AUC_{0-10h} ratio Day 14/baseline = 0.93; 90% CI 0.86–1.00). As expected, UGE was higher in patients on concomitant treatment of metformin and ipragliflozin (mean \pm SD Ae_{0-24h} glucose Day 14 was 74.9 ± 31.5 g) compared with patients on metformin and placebo (mean \pm SD Ae_{0-24h} glucose Day 14 was 3.56 ± 4.0 g). **Conclusion:** Multiple dosing of ipragliflozin 300 mg qd for 2 weeks was safe and well tolerated in T2DM patients on stable metformin therapy, with no signs of hypoglycemia observed. Combination treatment with ipragliflozin and metformin did not result in clinically relevant changes in AUC of metformin. UGE clearly increased in T2DM patients on concomitant treatment of metformin and ipragliflozin compared with patients on metformin and placebo.

Clinical Trial Registration Number: NCT01302145

850

The sodium glucose co-transporter-2 (SGLT2) inhibitor, PF04971729, provides multi-faceted improvements in diabetic patients inadequately controlled on metformin

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Background and aims: PF04971729 is a new, highly potent and selective SGLT2 inhibitor in development for treatment of type 2 diabetes mellitus (T2DM). This phase 2, randomized, placebo-controlled, double-blind, double-dummy study investigated the efficacy and safety of PF04971729 in patients with T2DM standardized on background of metformin ahead of randomization.

Materials and methods: A total of 328 patients (35% female) with T2DM and baseline characteristics (mean) of T2DM duration 6.3 years, HbA1c 8.1%,

body weight (BW) 83.8 kg, and blood pressure of 126/79 mmHg were randomized, to once daily doses of placebo, PF04971729 1 mg, 5 mg, 10 mg and 25 mg, or sitagliptin 100 mg for 12 weeks. Efficacy endpoints were HbA1c, BW, fasting plasma glucose (FPG), systolic and diastolic blood pressure (sBP and dBP) and fasting plasma insulin (FPI). Assessments of safety and tolerability were also included.

Results: Significant changes from baseline were observed in all parameters with PF04971729 as shown in the table below (analysis at Week 12, mean \pm se). The frequency of adverse events (AEs) by treatment group was numerically comparable across all treatment arms studies. The number of subjects withdrawn due to AEs was 9/328 (2.7%) and evenly distributed across all arms. Overall, there was no dose-related increase in frequency of AEs with increasing PF04971729 dose. There were no cases of pyelonephritis reported. The frequency of symptomatic urinary tract infections was 2.3% (PF04971729 arms combined) and 3.7% with placebo. The frequency of genital fungal infections was 3.6% pooled across PF04971729 doses versus 1.8% with placebo.

Conclusion: In patients with T2DM sub-optimally controlled on metformin, administration of once-daily PF04971729 appeared safe and well tolerated and resulted in meaningful, multi-faceted improvements in glycemic control, body weight, and blood pressure.

	Placebo	PF049717291 mg	PF049717295 mg	PF0497172910 mg	PF0497172925 mg	Sitagliptin100 mg
Subjects	54	54	55	55	55	55
Randomized						
HbA1c (%)	-0.11 \pm 0.11	-0.56 \pm 0.11 [^]	-0.80 \pm 0.11 [^]	-0.73 \pm 0.11 [^]	-0.83 \pm 0.11 [^]	-0.87 \pm 0.11 [^]
FPG (mg/dL)	2.76 \pm 4.08	-18.23 \pm 3.96 [^]	-23.06 \pm 4.01 [^]	-31.47 \pm 4.08 [^]	-29.26 \pm 4.14 [^]	-17.29 \pm 4.00 [^]
BW (%)	-0.75 \pm 0.34	-1.90 \pm 0.33 [^]	-2.50 \pm 0.33 [^]	-2.90 \pm 0.34 [^]	-2.66 \pm 0.34 [^]	-0.30 \pm 0.33
sBP (mmHg)	-0.55 \pm 1.56	-2.69 \pm 1.52	-4.03 \pm 1.53	-3.43 \pm 1.56	-3.93 \pm 1.57	-1.09 \pm 1.53
dBP (mmHg)	0.81 \pm 0.95	-1.12 \pm 0.92	-1.01 \pm 0.93	-3.18 \pm 0.95 [^]	-1.83 \pm 0.95 [^]	1.68 \pm 0.93
FPI (%) ^a	12.63 \pm 6.93	3.45 \pm 6.33 [^]	3.98 \pm 8.95	-14.91 \pm 5.37 [^]	-15.98 \pm 5.39 [^]	14.05 \pm 8.33

^a FPI reported as percent change from baseline for observed cases; all other parameters presented using last-observation-carried-forward analysis; [^] statistically significantly different (one-sided p-value < 0.05) compared to placebo

Clinical Trial Registration Number: NCT01059825

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Dapagliflozin added-on to pioglitazone is effective in improving glycaemic control and attenuates weight gain without increasing hypoglycaemia in patients with type 2 diabetes

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Background and aims: Dapagliflozin (DAPA), an SGLT2 inhibitor, acts independently of insulin secretion or action. In clinical trials, DAPA has been shown to reduce hyperglycaemia and improve glycaemic control in patients with type 2 diabetes (T2DM) alone or added-on to metformin, sulfonylurea or insulin. This report examines DAPA added-on to the thiazolidinedione (TZD), pioglitazone (PIO).

Materials and methods: The primary objective was HbA_{1c} change from baseline with DAPA+PIO versus placebo (PBO)+PIO at week 24 in patients with T2DM who were inadequately controlled on PIO alone having HbA_{1c} ≥ 7.0 – $\leq 10.5\%$. Following run-in periods of 2 weeks for those on previous PIO (48%) or of 12 weeks for those starting PIO (52%), patients on PIO ≥ 30 mg were randomised to DAPA 5 or 10 mg or PBO QD plus open-label PIO.

Results: Mean baseline demographics included: age 53.5 yr, duration T2DM of 5.5 yr, HbA_{1c} 8.4%, FPG 9.16 mmol/L, weight 86 kg, and 78% of patients had BMI ≥ 27 kg/m². Significant mean reductions occurred in HbA_{1c}, FPG, and 120-min PPG at week 24 and maintained through week 48 in both DAPA groups (Table). Body weight gain occurred in the placebo group while no meaningful change from baseline was noted in the DAPA 10 mg group. The adjusted mean changes from baseline for body weight were statistically significant for the DAPA 10 mg group at week 24 and maintained throughout week 48 (Table). Discontinuations were low and similar in DAPA and PBO groups. The proportion of patients reporting at least one AE was similar for the DAPA and placebo groups through week 48. Through 48 weeks, one patient on placebo, and three patients on DAPA 5 mg experienced hypoglycaemia episodes while no hypoglycaemia occurred in the DAPA 10 mg group. No episodes of major hypoglycaemia events were reported. Signs and symptoms suggestive

of genital infection were reported in 8.6–9.2% on DAPA versus 2.9% on PBO through 48 weeks. No clear drug effect was observed on the frequency of signs and symptoms suggestive of UTIs; the proportion of patients with signs and symptoms suggestive of UTI was 7.9% for placebo, 8.5% for DAPA 5 mg and 5.0% for DAPA 10 mg through 48 weeks.

Conclusion: In patients with T2DM who were inadequately controlled on TZDs, the addition of DAPA improved HbA_{1c}, FPG, and 120-min PPG. DAPA was well tolerated, and mitigated the weight gain of PIO without increasing hypoglycaemia risk.

Efficacy Results

	Week 24 (LOCF)			Week 48 ^a		
	PBO+PIO (n=139)	DAPA 5mg + PIO (n=141)	DAPA 10mg + PIO (n=140)	PBO+PIO (n=139)	DAPA 5mg + PIO (n=141)	DAPA 10mg + PIO (n=140)
HbA _{1c} , %	-0.42 (0.08)	-0.82 (0.08) ^b	-0.97 (0.08) ^b	-0.54 (0.08)	-0.95 (0.08)	-1.21 (0.07)
FPG, mmol/L	-0.31 (0.16)	-1.38 (0.16) ^b	-1.64 (0.16) ^b	-0.73 (0.20)	-1.26 (0.18)	-1.84 (0.17)
FPG, mg/dL	-5.5 (2.9)	-24.9 (2.9) ^b	-29.6 (2.9) ^b	-13.1 (3.6)	-22.8 (3.2)	-33.1 (3.0)
120-min PPG, mmol/L	-0.78 (0.36)	-3.61 (0.35) ^b	-3.75 (0.36) ^b	-1.41 (0.39)	-3.35 (0.33)	-4.49 (0.32)
120-min PPG, mg/dL	-14.1 (6.4)	-65.1 (6.3) ^b	-67.5 (6.4) ^b	-25.4 (7.1)	-60.4 (5.9)	-80.9 (5.7)
Weight, kg	1.64 (0.28)	0.09 (0.28) ^b	-0.14 (0.28) ^b	2.99 (0.41)	1.35 (0.38)	0.69 (0.36)

Adjusted mean change from baseline (SE); ^aThe primary analysis at week 24 was based on ANCOVA model using LOCF; all remaining data used repeated measure analysis. Data after rescue were excluded from the analysis; ^bp-value < 0.001

Clinical Trial Registration Number: NCT00683878

Supported by: BMS and AZ

852

Long-term efficacy and safety of add-on dapagliflozin vs add-on glipizide in patients with type 2 diabetes mellitus inadequately controlled with metformin: 2-year results

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Background and aims: Dapagliflozin (DAPA), a selective SGLT2 inhibitor, reduces hyperglycemia by increasing urinary glucose excretion in a manner independent of insulin secretion or action. In subjects with T2DM inadequately controlled on MET, 1-year results of a randomized, double-blind trial of DAPA (up to 10 mg/d; n=406) or glipizide (GLIP, up to 20 mg/d; n=408) added to open-label MET (median 2000 mg/d) have been reported (D1690C00004). The primary endpoint was change in HbA_{1c} at year 1 with 0.52% reduction in both arms. While DAPA was non-inferior to GLIP in reduction of HbA_{1c} at year 1, DAPA produced weight loss, and reduced risk of hypoglycemia compared with GLIP. We now report efficacy and safety over 2 years of treatment of DAPA+MET compared with GLIP+MET.

Materials and methods: In the year of double-blind extension, subjects continued to receive DAPA (n=315) or GLIP (n=309) added to MET. During the first 18 weeks of the first year of the study, medication was up-titrated based on FPG; a one-time up-titration was allowed in the second year of treatment. Glycemic efficacy and weight change were analyzed over 2 years by longitudinal repeated-measures analysis and reported as adjusted mean changes with 95% CI. Proportion of patients with ≥ 1 episode of hypoglycemia was calculated.

Results: Mean overall baseline HbA_{1c} was 7.72%. Entering year 2, 90.5% of DAPA and 73.1% of GLIP subjects were taking maximum doses. At the end of year 2, change in HbA_{1c} with DAPA was -0.32% (-0.42, -0.21) vs -0.14% (-0.25, -0.03) with GLIP. Body weight reduction seen at year 1 with DAPA was sustained through year 2: -3.70 kg (-4.16, -3.24) vs +1.36 (0.88, 1.84) for GLIP. Proportion of subjects with ≥ 5% weight loss was 23.8% with DAPA and 2.8% with GLIP. Dapa had low risk of hypoglycemia over 2 years: 4.2% vs GLIP 45.8% of subjects experienced ≥1 episode of hypoglycemia. Systolic blood pressure was reduced with DAPA, -2.7 mm Hg (-4.2, -1.2) vs +1.2 mm Hg (-0.4, 2.8) with GLIP. The overall rate of AEs remained similar between arms over 2 years. On active questioning, the proportion of subjects reporting signs, symptoms, and events suggestive of UTI was 13.5 % for DAPA and

9.1% for GLIP and the proportion of subjects reporting signs, symptoms, and events suggestive of genital infections was 14.8% for DAPA (8.0% in men, 23.3% in women) and 2.9% (0.4% in men, 5.9% in women) for GLIP over 2 years. The majority of events occurred in year 1, were mild to moderate in intensity, and responded to standard care. One discontinuation in each arm due to UTI and 3 discontinuations in the DAPA arm due to genital infections occurred during year 1; no discontinuations due to UTI or genital infections were reported during year 2. There was no relevant change in renal function measured by eGFR over 2 years.

Conclusion: DAPA treatment in patients with T2DM inadequately controlled on MET showed sustained glycemic efficacy and weight loss with low risk of hypoglycemia over a 2-year period compared with GLIP. Higher frequencies of events suggestive of genital infections and UTI were reported, mainly in the first year. These responded to standard treatment and rarely led to discontinuation.

Clinical Trial Registration Number: NCT00660907

Supported by: AstraZeneca and Bristol-Myers Squibb

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Dapagliflozin, a selective SGLT2 inhibitor, has a low propensity to cause hypoglycaemia in patients with type 2 diabetes

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Background and aims: Hypoglycaemia is associated with recurrent morbidity in patients with type 2 diabetes mellitus (T2DM), and is often a barrier to achieving glycaemic targets. Dapagliflozin (dapa), a selective inhibitor of renal sodium-glucose cotransporter 2 (SGLT2), reduces hyperglycaemia in patients with T2DM by increasing urinary glucose excretion. This effect depends on baseline glycaemic control and renal filtration rate, but is independent of insulin secretion or action. Dapa reduces the glucose load filtered by the kidney without affecting other mechanisms which maintain glucose homeostasis, suggesting that dapa may possess a low intrinsic propensity for hypoglycaemia.

Materials and methods: Hypoglycaemic episodes (hypos) were reviewed from four placebo- and one active-controlled Phase 3 studies of dapa in T2DM (Table). In the dapa added to insulin study (NCT00673231), insulin dose could be up-titrated or down-titrated, and in the dapa versus glipizide study (NCT00660907), both treatments were up-titrated and could be down-titrated to achieve glycaemic control. In the dapa added to glimepiride study (NCT00680745), down-titration of the sulfonylurea was permitted in the event of recurrent hypos. In practice, protocol-based guidance on down-titration was not fully applied in these studies. In the remaining studies, treatments remained constant. Total and major hypos were defined as described in the table. Study discontinuations as a result of hypos were also reviewed.

Results: When dapa was used alone or added to metformin, there were no more hypos than placebo, but when added to glimepiride or insulin, there were more hypos than with placebo. In the active comparison study, hypos were more than tenfold less frequent with dapa (3.4%) versus glipizide treatment (39.7%).

Conclusion: The low hypoglycaemic frequency when used alone, or when added to metformin at a fixed dose or titrated, suggests that dapa has a low intrinsic propensity to cause hypoglycaemia in patients with T2DM. Although hypos were more frequent compared with placebo when dapa was added to background glimepiride or insulin, this effect is typically observed in clinical trials where, unlike normal clinical practice, agents with low hypoglycaemic propensity are added to stable doses of a sulphonylurea or insulin.

Hypoglycemia in studies of dapagliflozin in T2DM patients with inadequate glycaemic control

Study ID Design Time point	Treatment group	Patients with ≥ 1 hypoglycaemic episode, x/n (%)		
		Total	Major	Leading to discon
Placebo-controlled studies				
NCT00528372 Monotherapy 24 weeks	Placebo	2/75 (2.7)	0	0
	DAPA 2.5 mg QAM	1/65 (1.5)	0	0
	DAPA 5 mg QAM	0/64	0	0
	DAPA 10 mg QAM	2/70 (2.9)	0	0
	DAPA 2.5 mg QPM	1/67 (1.5)	0	0
	DAPA 5 mg QPM	0/68	0	0
	DAPA 10 mg QPM	1/76 (1.3)	0	0
NCT00528879 Add-on to metformin 102 weeks	Placebo	8/137 (5.8)	0	0
	DAPA 2.5 mg	5/137 (3.6)	0	0
	DAPA 5 mg	7/137 (5.1)	0	0
	DAPA 10 mg	7/135 (5.2)	0	0
NCT00680745 Add-on to glimepiride 48 weeks	Placebo	10/146 (6.8)	0	0
	DAPA 2.5 mg	15/154 (9.7)	1/154 (0.6)	0
	DAPA 5 mg	15/145 (10.3)	0	0
	DAPA 10 mg	17/151 (11.3)	0	0
NCT00673231 Add-on to insulin \pm other OADs 48 weeks	Placebo	102/197 (51.8)	2/197 (1.0)	0
	DAPA 2.5 mg	122/202 (60.4)	3/202 (1.5)	0
	DAPA 5 mg	118/212 (55.7)	2/212 (0.9)	0
	DAPA 10 mg	105/196 (53.6)	3/196 (1.5)	0
Active comparison, titration study				
NCT00660907 Add-on to metformin 52 weeks	Glipizide ≤ 20 mg*	162/408 (39.7)	3/408 (0.7)	6/408 (1.5)†
	DAPA ≤ 10 mg*	14/406 (3.4)	0	0

T2DM = type 2 diabetes mellitus. x = number of patients with a hypoglycaemic episode. n = number of patients in each treatment group in study safety analysis datasets. Total = total hypoglycaemic episodes. Major = symptomatic episodes requiring external assistance due to severe impairment in consciousness or behaviour with a capillary or plasma glucose <3.0 mmol/L (54mg/dL) and prompt recovery after glucose or glucagon administration. discon = study discontinuation. DAPA = dapagliflozin. QAM = once daily administration in the morning. QPM, once daily administration in the evening. OAD = oral antidiabetic drug.; *mean titrated dose 16.4mg for glipizide and 9.2mg for dapagliflozin. †Patients with inadequate glycaemic control at maximum dose were discontinued from the study.

Supported by: AstraZeneca and Bristol-Myers Squibb

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Changes in insulin sensitivity as measured by glucose disposal rate and acute insulin secretion with the sodium glucose co-transporter 2 inhibitor dapagliflozin

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Table									
PBO N=21					DAPA N=23				
n	Mean Baseline	Mean at 12 wks LOCF	Adjusted Mean Percent Change from Baseline at 12 wks LOCF (a)		n	Mean Baseline	Mean at 12 wks LOCF	Adjusted Mean Percent Change from Baseline at 12 wks LOCF (a)	Between Group Adjusted Mean Difference
GDR (SE) mg/kg/min	19	6.85 (1.838)	6.36 (1.510)	-9.99 (4.0392)	22	7.95 (2.041)	8.21 (2.005)	7.98 (4.4849)	19.97 (7.4685) P= 0.0059
(a) Based on ANCOVA model for (log (Week 12 LOCF value)- log (baseline value)) with treatment group as an effect and log (baseline value) and prior anti-diabetic medication use as a covariate.									
	Mean Baseline	Mean at 12 wks LOCF	Adjusted Mean Percent Change from Baseline at 12 wks LOCF (b)		Mean Baseline	Mean at 12 wks LOCF	Adjusted Mean Percent Change from Baseline at 12 wks LOCF (b)		
AIRg (SE) mU/L*min	19	25.37 (30.54)	17.01 (30.58)	-12.73 (10.71)	23	41.37 (56.05)	54.76 (71.63)	15.39 (9.59)	28.13 (14.50) P= 0.0598
(b) Based on ANCOVA model for Week 12 LOCF value - baseline value with treatment group as an effect and baseline value and prior anti-diabetic medication use as a covariate.									
	Mean Baseline	Mean at 12 wks LOCF	Mean change from baseline at 12 wks LOCF		Mean Baseline	Mean At 12 wks LOCF	Mean change from baseline at 12 wks LOCF		
HbA1c (SD) %	20	7.56 (0.65)	7.59 (1.208)	0.03 (1.088)	23	7.51 (0.802)	7.13 (0.606)	-0.38 (0.516)	Not estimated
Body weight (SD) kg	21	98.95 (15.341)	99.53 (15.252)	0.62 (2.783)	23	99.76 (22.574)	98.17 (22.236)	-1.58 (2.960)	Not estimated

N= Total number of patients who took at least one dose of double-blind study medication, n= Number of patients with non-missing baseline and week 12 LOCF values

Background and aims: Inhibition of the sodium glucose co-transporter 2 (SGLT2), the predominant glucose transporter in the proximal tubule of the kidney, has been shown to improve glycaemic control in patients with type 2 diabetes (T2DM) independently of insulin secretion or action. Treatment with dapagliflozin (DAPA), an oral and selective SGLT2 inhibitor under development for the treatment of T2DM, is associated with reductions in hyperglycaemia and modest reductions in body weight (BW), which both have the potential to improve insulin sensitivity and secretion. This study was designed to evaluate changes in these parameters with DAPA.

Materials and methods: This 12 wk randomized, double-blind, placebo-controlled, parallel-group study aimed to assess the effects of once daily 5 mg DAPA on insulin sensitivity as measured by glucose disposal rate (GDR) in subjects with T2DM. Forty four subjects (29 male, 15 female; 36-69 yrs; mean T2DM duration 8.27 ± 6.03 yrs; mean HbA1c 7.52 ± 0.73 %; mean BW 99.38 ± 19.24 kg) on metformin therapy with or without an insulin secretagogue were randomized to receive either DAPA 5 mg QD or matching placebo (PBO). During the 12-wk treatment period, subjects maintained stable doses of background antidiabetic medication. GDR, calculated using the tracers methodology during the last 40 min of a 5 h hyperinsulinemic euglycaemic glucose clamp (HEC), was used as a measure of insulin sensitivity. Insulin secretion was determined as the acute insulin response to glucose (AIRg) during the first 10 min of a frequently sampled intravenous glucose tolerance test.

Results: An increase from baseline (BL) in adjusted mean GDR at Wk 12 was observed with the DAPA group that was significantly different from the PBO group, which showed a decrease. No correction was made for urinary glucose loss, though a sensitivity analysis accounting for urinary glucose loss, measured during the basal step of the HEC, showed similar results. An adjusted mean increase in AIRg at Wk 12 was observed with DAPA treatment relative to BL compared to an adjusted mean decrease with PBO. The difference between the two groups did not reach statistical significance. Consistent with previous studies, a mean reduction from BL in HbA1c was observed at Wk 12 in the DAPA group compared to a slight increase in the PBO group. In addition, mean reductions in BW were observed at Wk 12 in the DAPA group compared with an increase in the PBO group. DAPA was well tolerated by subjects in this study. No subjects in the PBO group and 1 in the DAPA group had hypoglycaemic events, none of which were major or led to treatment discontinuation.

Conclusion: Significant improvement in overall GDR compared with PBO was observed over 12 wks of treatment with the SGLT2 inhibitor DAPA in subjects with T2DM.

Clinical Trial Registration Number: MB102045/NCT00831779

Supported by: BMS and AZ

PS 072 Diabetes in childhood

855

Insulin resistance rises from mid-childhood, before the onset of puberty: longitudinal data from The EarlyBird Study

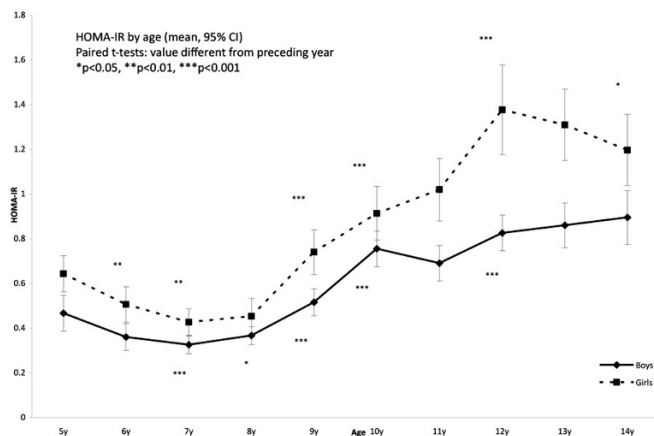
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Background and aims: Insulin resistance is key to the development and control of diabetes. It is known from cross-sectional studies that insulin resistance is higher in puberty, but longitudinal studies are needed to establish at what age the rise begins. Our aim was to document the behaviour of insulin resistance in healthy children aged 5–14y.

Materials and methods: 246 healthy children (141 boys) from the EarlyBird cohort, monitored annually from 5 to 14 years (y) for insulin resistance (HOMA-IR), %fat (DEXA), and self-assessed Tanner Stage (TS; mean genital/breast and pubic hair development). Onset of puberty was defined as TS 2.

Results: The figure shows trends in mean HOMA-IR from 5 to 14y. Insulin resistance began to rise at 7y in boys, 8y in girls. In a linear mixed effects model, insulin resistance rose in boys by 18% (95% CI 15%, 22%), girls 15% (12%, 18%; both $p < 0.001$) over the 4 year period leading up to the onset of puberty, independent of changes in %fat. Insulin resistance continued to rise during puberty in both genders (in girls until TS 4, boys until TS 3, both $p < 0.001$).

Conclusion: The 'pubertal' increase in insulin resistance begins well before pubertal onset, independently of increasing adiposity. The cause of this mid-childhood rise is unclear, but may relate to adrenarche, which typically occurs at 6 to 8y. The period of increased risk for diabetes development, and for increased insulin requirement in those with established diabetes, extends from adolescence back to mid-childhood.



Supported by: NNUKRF, EBDT, BFT, Nestec, PMS

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Metabolic consequences of hepatic steatosis in overweight youth

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Objective: To determine the association between hepatic steatosis and metabolic risk factors for type 2 diabetes mellitus in adolescents.

Study design and methods: This was a cross-sectional study of 98 overweight adolescents and 11 healthy weight controls aged 13–18yrs. The primary exposure variable was hepatic steatosis defined as hepatic triglyceride ≥ 5.5 mg/g tissue, determined with ¹H-magnetic resonance spectroscopy. The main outcome measures were insulin sensitivity measured by frequently sampled intravenous glucose tolerance test, the presence of the metabolic syndrome, glucose response to an oral glucose challenge and disposition index as a measure of beta cell function. Control variables included visceral fat mass measured with magnetic resonance imaging, physical activity with daily step counts and cardiorespiratory fitness measured with expired gases at the end of the progressive exercise test to exhaustion.

Results: Hepatic steatosis was evident in 31% of overweight adolescents and was associated with lower insulin sensitivity ($p = 0.02$) and a higher prevalence of the metabolic syndrome ($p = 0.001$) compared to youth without steatosis. Clinical features included a higher BMI z-score ($p = 0.02$), and waist circumference ($p = 0.003$). After matching cases and controls for age, sex, and visceral fat mass, those with hepatic steatosis displayed lower insulin sensitivity (3.04 vs 5.10, $p = 0.011$), higher rates of the metabolic syndrome (50 vs 14%, $p = 0.034$), and increased glucose area under the curve (842 vs 754 $p = 0.040$). These differences were not evident when youth were stratified according to visceral fat mass and matched for hepatic triglyceride content. No differences were observed in physical activity, cardiorespiratory fitness or beta cell function between cases with hepatic steatosis and controls with normal hepatic triglyceride content. In multivariate regression analyses, hepatic triglyceride content was a significant predictor of insulin sensitivity ($B = -0.24$, $t = -2.29$, $p < 0.025$), the metabolic syndrome ($B = -0.54$, $t = -5.8$, $p < 0.001$), and glucose area under the curve ($B = 0.33$, $t = 3.3$, $p < 0.001$) independent of visceral and whole body adiposity.

Conclusion: We provide novel evidence that hepatic steatosis is an early biomarker for T2DM in overweight adolescents independent of visceral and whole body adiposity.

Supported by: CIHR and Lawson Foundation

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Prevalence and risk factor of childhood overweight and obesity in primary school children of Dhaka city

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Background and aims: Childhood obesity has become a serious public health problem because of its strong association with adulthood obesity and the related adverse health consequences including development of diabetes. Epidemiological data on childhood obesity is virtually nonexistent in Bangladesh. Obesity is strongly associated with insulin resistance, which, when coupled with relative insulin deficiency, leads to the development of overt type 2 diabetes mellitus. The aim of the present study is to determine the prevalence of childhood overweight and obesity and its risk factors in Bangladeshi urban primary school children.

Materials and methods: A cross-sectional study was conducted in primary school children. This study included 1200 participants (both boys and girls aged 6 to 13 years old) collected by following a simple random procedure. On the basis of predefined scoring, subjects were classified as underweight, normal, overweight and obese if their weight-for-height values were within the following ranges of the recommended median values (<90.99% under weight, 91–110% normal weight, 111–120% overweight and > 120% obese). Univariate multinomial regression models were used to estimate the odds ratios (ORs) and the 95% confidence intervals (95% CI). Multivariate multinomial regression models were used to estimate adjusted OR and the 95% CI for three categories of weight-for-height.

Results: The prevalence of overweight was found to be 13.2% (95% CI: 11.33 – 15.17) and obesity 17.8% (95% CI: 15.59 – 19.91) among the study subjects. The prevalence of overweight and obesity among boys were 13.6% (95% CI: 10.83 – 16.41), 22.1% (95% CI: 18.69 – 25.45) and among girls these were 12.9% (95% CI: 10.26 – 15.54) and 13.7% (95% CI: 11 – 16.42), respectively. There were significant difference found in weight for height ($p = 0.015$) and weight ($p < 0.001$) between boys and girls. Mean fat intake of the obese group was significantly higher in overweight and underweight group ($p = 0.008$). The odds for obesity were higher for boys than their counterparts. The study showed that associated risk factor for developing obesity was 1.65 times higher for boys compare to the girls ($p = 0.003$ and CI% 1.19–2.29). The household monthly income and parents education has a significant risk for obesity, even after being adjusted with, age, income, parent's education and physical activities compared to the reference groups. Mean fat intake of the obese group was significantly higher compared to the overweight and underweight groups ($p = 0.008$). Fat intake was significantly and positively associated with the children's weight for height.

Conclusion: The data suggest a high prevalence of overweight and obesity in urban primary school children in Bangladesh. Positive energy balance with higher fat intake, high income of the family and higher education level of the parents (probably reflecting higher socioeconomic class) seem to be among

the major underlying factors for the increased prevalence of childhood obesity in this society.

Supported by: BADAS, NOMA Grant

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Prevention of diabetes and obesity in children and teenagers through the Coubertin educational model

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Background and aims: Bad eating habits, little exercise and a sedentary life style lead to overweight and obesity among children and teenagers. The later risk factors of diabetes at an adult age. That is why, the 2004 Geneva Declaration of the WHO suggested actions to be carried out in the school setting for the prevention of diabetes and obesity. In August 2005, the Coubertin Educational Model (Oaxaca, México) was set off. Based on both constructivism and humanism, this educational method integrates academic, sport, nutritional and psychological instruction seeking to promote life-lasting habits (a balanced diet, exercise and a healthy life style) that lead to the prevention of obesity, diabetes and associated illnesses.

Material and methods: 675 students were included (454 of elementary and 221 of junior high), from different school years (SY): 70 from 2005–2006, 195 from 2006–2007, 131 from 2007–2008, 122 from 2008–2009 and 157 from 2009–2010. At the beginning and end of each school year both medical and physical aptitude tests were carried out. Along the school cycle, students took daily sport sessions structured in such a way that they were strictly evaluated and graded. Students would also get medical, nutritional and sport assistance. The cafeteria had a strong control on the quality of the food provided to the kids.

Results: In the 2005–06 school year the prevalence of obesity went down from 18.5% to 7.1% and that of overweight fell from 20.0% to 14.2%. In the 2006–07 SY, obesity decreased from 14.2% to 5.7% and overweight went down from 18.6% to 7.2%. In the 2007–08 school year, obesity moved from 16.6% to 6.2% and overweight diminished from 27% to 16.6%. Next school year, 2008–09 obesity started at a rate of 13.2% and finished at a rate of 4.5% while overweight cut down almost by half by going from 14.2% to 7.6%. Last school year, 2009–2010 obesity started from some 10% and came down to only 2.5% whereas overweight flew from 12% to 5%. When the 2005 school year began 70% of the students were consuming too much sugar, 58.57% were eating an excess of fats while only 24.28% of them were including enough vegetables in their daily diets. Soda was a main source of liquids in 80% of the students. Finally, around two thirds of the pupils (62.85%) watched TV for more than 3 hours a day in contrast with just a third (31.42%) who practiced a regular physical activity three or more times a week. By 2010 changes in that group are outstanding: Only 9.2% of the students keep having an excess of sugar in their diets whereas 14.0% continue consuming too much fat. In contrast, all of them (100%) include vegetables in their menus nowadays and none drink soda. Finally, just a fifth (19.14%) spends more than three hours a day in front of the TV set while all of them (100%) exercise three or more times a week. Facts for the 2006, 2007, 2008 and 2009 groups are about the same.

Conclusions: In this study, the Coubertin Educational Model established good eating habits, regular exercise and a healthy life style which brought down both obesity and overweight among students and therefore proved to be an effective way to reduce the risk of diabetes.

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HbA_{1c} does not detect glucose impairment in youth

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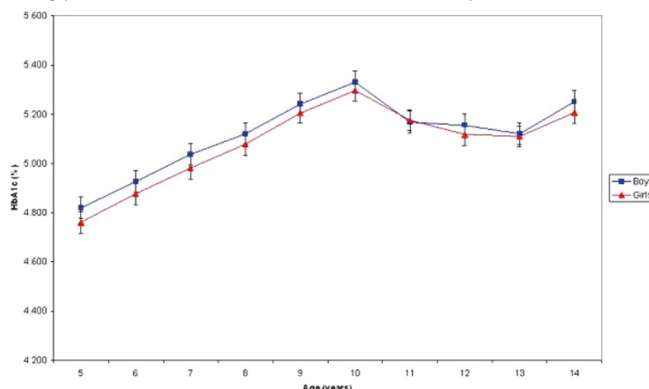
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Background and aims: An HbA_{1c} threshold of $\geq 6.5\%$ has been adopted by the ADA to diagnose diabetes in adults, and of $\geq 5.7\%$ to identify individuals at risk. Little, however, is known of HbA_{1c}'s behaviour or diagnostic value in youth. Our aim was to evaluate the diagnostic value of HbA_{1c} in youth at risk of diabetes.

Materials and methods: HbA_{1c} (DCCT aligned) and glucose were measured annually in 274 children from the age of 5y to 14y. Both cross-sectional association and longitudinal trends were examined. ROC analysis was used to determine the diagnostic value of HbA_{1c} in those individuals who had impaired fasting glucose (IFG - glucose $\geq 5.6\text{mmol/l}$).

Results: Cross-sectionally, there were positive associations between HbA_{1c} and glucose ($r = 0.11 - 0.30$, $p = 0.07 - < 0.001$). However, from age 10y HbA_{1c} fell (Figure) while glucose rose ($p < 0.001$). Some 64 observations recorded IFG between 10 and 14y, but HbA_{1c} exceeded 5.7% in only four of them. The area under the ROC curve was modest at 0.67 ($p < 0.001$). Sensitivity and specificity for predicting IFG were optimal at 52% and 76% respectively, corresponding to an HbA_{1c} of 5.4%.

Conclusion: HbA_{1c} is not diagnostically useful in youth at risk of diabetes (IFG). Although HbA_{1c} retains a positive association with glucose over time, their trends from 10y are opposite in direction, suggesting that factor(s) beside glycaemia influence(s) the variance of HbA_{1c} in youth.



Supported by: NNUKRE, EBDT, BFT, Nestec, PMS

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Intensified insulin therapy: an additional burden for young children with type 1 diabetes and their families?

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Objective: Compare quality of life of young children with type 1 diabetes (T1DM) and their parents' well-being with intensified (IT:CSII + ICT) or conventional insulin (CT) therapy.

Methodology: In a cross sectional study, all children of 18 centres in North America Australia, Europe and Japan, age <11 yrs with a diabetes duration (DD) ≥ 1 y, and their parents, were invited to participate. Information on clinical characteristics, treatment, severe hypoglycaemia and diabetic ketoacidosis were collected. HbA_{1c} was measured centrally by ¹⁸Tosoh liquid chromatography (DCCT aligned normal range 4.4–6.3%). Quality of Life (QoL) was evaluated by kidscreen index (≥ 7 years); kidscreen-27 proxy (physical well-being, psychological well-being, autonomy and parent relation, social support and peers, school) and WHO-5 for parents.

Results: In total 1133 children participated: IT (n=562, 47.4% F, age 8.1 ± 2.1 y, CT n=570; 48% F; age 7.9 ± 2.1 y). Diabetes duration in the IT was significantly longer (3.6 ± 2.9 vs 3.1 ± 2.0 yrs) with a significantly lower HbA_{1c} (7.9 ± 0.9 vs 8.1 ± 1.1 %) and less hypoglycaemic events (.02 vs .05). Children with diabetes overall do not differ from norm data of healthy peers in any of the QoL dimensions. A significantly better QoL was reported in the IT group ($< .02$) on all subscales of kidscreen questionnaire, except psychological well being. Parent well-being did not differ between the two groups.

Conclusion: In this large international crosssectional study, IT in young children with T1DM results in an improved metabolic outcome, less hypoglycaemia and in 4/5 dimensions better quality of life, suggesting that intensification of therapy can be started early onwards.

Supported by: Novo Nordisk

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Evaluation of 3 bolus calculators in children and adolescents with type 1 diabetes using insulin pump therapy

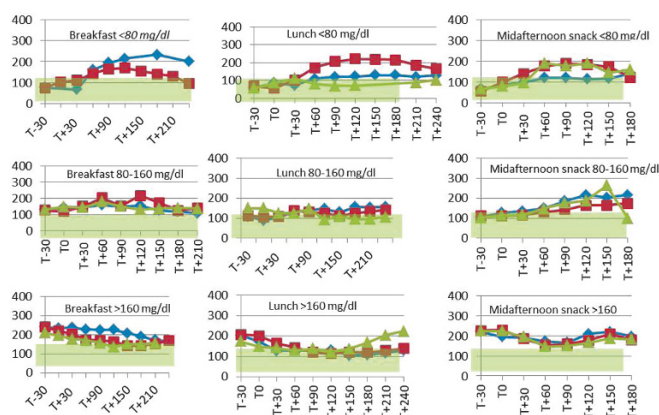
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Background and aims: Recently, pump manufacturers have engineered a new feature called 'bolus calculator', which calculates bolus insulin doses based on input from the pump wearer, which functions to help patients obtain optimum control over blood glucose levels. The bolus calculator takes into account the patient's current blood glucose, target blood glucose, amount of carbohydrate consumed, and other factors such as insulin sensitivity and insulin-to-carbohydrate ratio as well as duration of insulin action. Each pump company calculates insulin doses in a slightly different way. The aim of this study is to compare the efficacy of three bolus calculators (Animas 2020, Animas, Accu-Chek Combo, Roche, Paradigm Veo, Medtronic) to safely reduce postprandial hyperglycemia in children and adolescents with type 1 diabetes using insulin pump therapy.

Materials and methods: We enrolled 22 patients, aged 9–22 yrs. (mean 16.2 ± 4.9 yrs.) with type 1 diabetes from 1 to 18 yrs. (9.1 ± 4.1 yrs.), BMI 21.9 ± 4.5 kg/m², using insulin pump therapy for more than 6 months (insulin requirement 0.86 ± 0.27 U/kg/day, HbA1c $8.3 \pm 0.9\%$). For each patient we studied on 3 occasions (order of bolus calculator used randomly assigned) 3 different meals (breakfast, lunch and midafternoon snack); their composition was exactly the same in terms of quality and quantity on the 3 days. Insulin bolus, was injected 15 min prior meal. Patients checked glycaemia 30 minutes before meal and every 30 minutes for the following 4 hours. In case of hyperglycaemia after 2 hours from meal, a correction bolus was calculated using the bolus calculator assigned for that day.

Results: Data are shown in the table. The performance of the 3 bolus calculators was similar and did not show any statistical difference among them ($p=0.278$ by ANOVA). In case of pre-prandial hyperglycaemia or hypoglycaemia, Medtronic bolus calculator showed the best performance on average when compared with both Animas and Roche bolus calculators ($p=0.003$ for hypo and $p=0.041$ for hyper, respectively).

Conclusion: Bolus calculator seems a good feature to improve insulin pump therapy performance in children and adolescents with type 1 diabetes. No significant difference has been observed among the 3 different ones tested. However, when in hyperglycaemia, it is wise to separate the correction bolus from the meal bolus (to be injected 30 min prior the meal, and 15 to 20 min prior the meal bolus). Table - Glycaemic values (mg/dl) after using 3 different bolus calculator (Animas, A n=22, Medtronic, M n=22, and Roche, R n=18) after breakfast (B), lunch (L) and mid-afternoon snack (MS), according to different pre meal glycaemic ranges. *by ANOVA; ** $p=0.003$ by Kruskal-Wallis (among groups); $p=0.041$ by Kruskal-Wallis (among groups).



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Effectiveness and safety study of the prototype 4th generation seven day continuous glucose monitoring system in youth with type 1 diabetes mellitus

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Background and aims: Real-time continuous monitoring systems have been available to obtain better glycemic control in adult patients with diabetes. However, youth with type 1 diabetes are thought to have rapid glucose changes and to bring more challenge to the system user interfaces. The purpose of this study is to evaluate the effectiveness and safety of a prototype 4th generation of 7 day CGM system when worn by youth (6 to <18 years-old) with type 1 diabetes mellitus.

Materials and methods: 72 youth at 3 US centers were studied to test the performance of the prototype 4th generation 7 day CGM system. Subjects were scheduled for three consecutive 7-day wear periods (new sensor for each period, one masked and two unmasked periods). The OneTouch® Ultra2® meter was used for CGM system calibrations and diabetes management and subjects were instructed to use the CGM system information as an adjunct to their blood glucose (BG) meter readings. During the unmasked home use period, subjects were provided CGM glucose values and trend graphs and were asked to confirm high and low system alerts/alarms by taking a BG reading immediately after receiving a CGM alert/alarm.

Results: 70 subjects (97%) completed all visits. 37 subjects (51.4%) enrolled were male; 98.6% were White. The mean \pm SD (range) age of the enrollment population studied was 12.6 ± 2.8 (6.1 to 17.6) yrs. The mean \pm SD duration of diabetes was 6.3 ± 3.7 yrs. 59 subjects (81.9%) used CSII for insulin therapy; 13 subjects (18.1%) were on MDI therapy. The mean \pm SD baseline HbA1C was $8.26 \pm 1.49\%$. Over three 7-day wearing periods, there were no serious adverse events, sensor fractures, or infection complications. Except for infrequent mild (12.0%) or moderate (1.2%) skin irritations at the sensor insertion and adhesive sites, no other device related adverse events were occurred. 82% sensors lasted till day 6 and 74% sensors lasted till day 7. A total of 6466 paired CGM sensor and meter glucose values were obtained. 75.6% (95%CI: 72.7%-78.4%) of CGM sensor measurements were within 1.1 mmol/l of SMBG (for value ≤ 4.4 mmol/l), and 74.4% (95% CIs: 73.2%-75.5%) of CGM sensor measurements were within 20% of SMBG (for value > 4.4 mmol/l). The overall mean and median Absolute Relative Differences (ARD) vs. SMBG were 16.3% and 12.0%, respectively. During the unmasked phase and based on the subject's BG results, the true alert rates were 88.3% at the high alert level of 11.1 mmol/l and 67.0% at the low alert level of 4.4 mmol/l. There was a statistically significant improvement in time spent in euglycemia (3.9–10.0 mmol/l) for the two weeks when sensor readings were unmasked with an average improvement of 1.2 hrs/day (95%CI: 0.6–1.9 hrs/day, $p<0.001$).

Conclusion: The study concludes that the duration of diabetes, age, sex and BMI showed no significant impacts on the system performance and there were no significant system performance differences between study centers or with masked vs. unmasked wear. The accuracy, alert rates, and sensor life of the prototype 7 day CGM in youth were similar to that of adult patients and compared favorably to the CGM system currently approved for pediatric use. Youth with type 1 diabetes had improved time spend within target range even with a short term CGM display wear.

Clinical Trial Registration Number: NCT01185496

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Association of epilepsy and type 1 diabetes in children and adolescents

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Background and aims: An association between diabetes and idiopathic generalized epilepsy has been described in adult patients. The aim of the investi-

gation was to estimate the prevalence of epilepsy and possible risk factors in diabetic children and adolescents.

Materials and methods: In an observational cohort study based on the DPV-database (German/Austrian DPV initiative) data from 45 851 patients (52 % male) with type 1 diabetes (<20 years) could be analyzed. The mean age was 13.9 ± 4.3 years (mean \pm SD) and the mean duration of diabetes 5.4 ± 4.2 years. To ensure that all possible epilepsy cases were identified, the data base was searched for the concomitant diagnosis epilepsy or epileptic convulsions and for antiepileptic medication.

Results: 705 patients with epilepsy were identified giving a prevalence of 15.5/1000. 375 patients were treated with antiepileptic medication, while 330 were without anticonvulsive therapy. Patients with epilepsy were younger at onset of diabetes (7.7 ± 4.1 vs 8.5 ± 4.3 yrs, $p < 0.01$) and shorter than patients without epilepsy (height-SDS -0.22 ± 1.28 vs -0.05 ± 1.04 , $p < 0.05$), while weight and BMI were comparable. Comparing patients with epilepsy currently on antiepileptic drugs (AED) with patients without AED height reduction was primarily observed in the patients on AED treatment (height-SDS -0.47 ± 1.26 vs $+0.05 \pm 1.1$, $p < 0.01$). No difference between patients with and without epilepsy could be demonstrated for metabolic control, type of insulin treatment, insulin dose and prevalence of B-cell specific autoantibodies. The risk for DKA was almost double in patients with epilepsy compared to patients with type 1 diabetes alone (DKA per 100 patient years 10.5 ± 1.46 vs 5.6 ± 0.14 , $p < 0.01$).

Conclusion: Diabetic children and adolescents show an increased prevalence of epileptic seizures compared to reported prevalence rates in non-diabetic children. Due to unknown reasons there seems to be an association between DKA risk and epilepsy in diabetic children and adolescents.

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Direct diabetes-related costs in young patients with early onset and long-lasting type 1 diabetes

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Background and aims: The incidence of type 1 diabetes (T1DM) is increasing worldwide, especially in younger children. Children with early onset of diabetes bear a greater life-time risk of developing diabetic complications and may cause higher costs than average. The aim of this study was to estimate diabetes-related direct costs of pediatric T1DM and their predictors in young German patients with early onset (0–4 years) and at least ten years diabetes duration.

Materials and methods: Data of 1,473 patients with diabetes onset at the age of 0–4 years during the period 1993–1999 and continuous documentation of treatment during 2006–2008 were included. Patients were 53% male, mean age (SD) was 13.42 (2.21) and mean diabetes duration (SD) 10.44 (1.94) years. Mean HbA1c of the study cohort was 8.1%. All diabetes-related health care services during the year 2007 were derived from a nationwide prospective documentation system (DPV). The study took the perspective of the statutory health insurance. For the analysis of direct diabetes-related costs, health care utilization was valued in monetary terms using inpatient and outpatient medical fees and retail prices. We estimated the association between total diabetes-related costs as well as different cost categories (dependent variables) and age, sex, diabetes duration, metabolic control, and migration background applying multiple regression models. Mean direct costs were compared to average costs of 12,001 prevalent T1DM patients less than 20 years of age and shorter diabetes duration (mean age: 12.1 years, mean duration: 4.7 years) in Germany.

Results: Mean diabetes-related direct costs were Euro 3,774 (quartile 1: 1,930, quartile 3: 4,988) per patient-year. Glucose-self monitoring was the main cost category (29.4%), closely followed by CSII (25.8%, pump and catheters), diabetes-related hospitalizations (21.9%), and insulin (18.2%). Other cost categories (diabetes-related ambulatory care, needles/syringes, glucagon sets, other medication) amounted each at most 2%. Total costs were 6.5% lower in males ($p=0.022$) and 8.9% lower in patients with migration background ($p=0.031$). They increased with poor metabolic control (HbA1c 7.5–9: +8.3% ($p=0.015$), HbA1c >9: +14.1% ($p=0.001$)). A diabetes duration >11 years compared to a shorter duration and an age >15 years versus 8–11 years did

not significantly affect total medical costs. However, costs were significantly higher in youths aged 12–15 years. Average costs were 7.6% higher than mean diabetes-associated costs of prevalent T1DM patients with shorter diabetes duration (Euro 3,507).

Conclusion: Female gender, pubertal age, and poor metabolic control were significantly associated with increased costs. Although average diabetes duration in this cohort was >10 years, mean diabetes-related direct costs were only slightly increased compared to costs in a cohort with shorter diabetes duration in Germany.

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PS 073 Nutrition and diet

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Children of higher income families eat 'better' and are taller, but they are metabolically less healthy

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Background and aims: It is a widely held belief that children from higher income families eat 'better', and are metabolically healthier as a result. However, like many such beliefs, the notion has largely gone untested. We have used objective measurement and fasting blood samples from a large cohort of children to test the relationships between body composition, metabolic health and diet. We hypothesised that children of higher income families would eat better, and would be slimmer and metabolically healthier as a result.

Materials and methods: Factor analysis (promax) was carried out on a 45-item food frequency questionnaire to establish the distribution of food choice (expressed as z-scores) among 307 9y-old children (n=170 boys, 137 girls) from the EarlyBird cohort. Height was measured in duplicate by stadiometer, % body fat by dual-energy x-ray absorptiometry (DEXA), subcutaneous fat by the sum of five skinfold thicknesses, and metabolic health by HOMA2-IR and serum leptin. The children were grouped according to family income.

Results: Two principal dietary components emerged, 'elemental' (E: fish, meat, vegetables, rice, pasta etc) and 'processed' (P: crisps, chips, sausage, chocolate, white bread etc.). 'E' foods were more frequently consumed by children of higher income families (mean difference = 0.44 z-scores, $p < .001$) and 'P' foods more frequently by children of lower income families (mean difference = 0.22 z-scores, $p < .001$). Consumption of 'E' foods was also associated with height. Thus, in a fully adjusted model controlling for family income, parental education and child gender, one unit (z-score) difference in 'E' food consumption was associated with 1.4 cm in height ($p < .05$). Taller children, however, were both fatter and metabolically less healthy. Accordingly, a unit difference in height was associated with 0.07 BMI SDS ($p < .001$), 0.60 % body fat ($p < .001$), 0.01 cm subcutaneous fat ($p < .001$), 0.03 units insulin resistance ($p < .001$) and 0.40 ng/ml serum leptin ($p < .001$).

Conclusion: Higher income families eat more elemental foods, and children who eat elemental foods are taller. Contrary to our hypothesis, however, such children tend to be fatter and metabolically less healthy. Protein over-nutrition among wealthier families may be one explanation.

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Prospective associations between dietary insulin index, glycaemic index and glycaemic load during puberty and body composition in young adulthood

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Background and aims: Puberty is a so-called critical period for overweight and characterized by physiological insulin resistance during mid-puberty. This study addressed the hypothesis that habitual consumption of a diet inducing higher levels of postprandial glycemia or insulinemia during puberty may have an unfavorable effect on body composition in young adulthood.

Materials and methods: Multivariate regression analysis was performed on 263 DONALD participants with at least two 3-day weighed dietary records during puberty (girls 9–14 years; boys 10–15 years) and anthropometric measurements in young adulthood (18–25 years). A published dietary glycemic index (GI) was assigned to each carbohydrate containing food. Similarly, each food was assigned a food insulin index (insulinemic response to a 1MJ portion of food relative to 1MJ of white bread) using 121 values measured at Sydney University.

Results: Dietary GI or glycemic load during puberty were not related to body composition in young adulthood. The dietary insulin index (II) during puberty was associated with higher levels of percentage of body fat (%BF) in young adulthood, even after adjustment for early life, socioeconomic and nutritional factors; %BF in energy-adjusted tertiles of dietary II were 23.1 (95%CI: 21.9, 24.4), 24.4 (23.2, 25.7), 24.8 (23.6, 26.0) (p for trend=0.02). Ad-

justment for baseline %BF attenuated this relationship (p for trend=0.1). Dietary II was not related to BMI.

Conclusion: This study suggests a prospective adverse influence of dietary II during puberty on body fat in young adulthood. Postprandial increases in insulinemia rather than increases in glycemia appear to be implicated in an unfavorable development of body composition.

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The role of glycaemic index and glycaemic load on weight loss and on the metabolic parameters of adults with type 2 diabetes

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Background and aims: The aim of the present study was to examine the effects of consumption of desserts with low glycaemic index (GI) and low glycaemic load (GL), as part of a balanced hypo-caloric diet, on glycaemic control and weight loss in patients with type 2 diabetes mellitus.

Materials and methods: A total of 48 overweight or obese adults with type 2 diabetes treated with oral antidiabetics were studied. The subjects were randomly assigned in two groups. The first group included 25 subjects [intervention group, mean age 61 ± 8 years, men/women (n) 9/16] and the second one 23 [control group, mean age 62.6 ± 7.6 years, men/women (n) 15/8]. Both groups followed the same hypo-caloric diet for three months under supervision of a clinical dietitian. The desserts of low GI/GL were included in the diet in the intervention group and they were allowed to consume 4 desserts/week, while no desserts were allowed in the control group. All participants underwent a complete clinical and biochemical evaluation at baseline and after the 3 months. Additionally, at the end of the three month period, subjects of both groups consumed in two different days two isocaloric desserts (a dessert of low GI/GL and a common high GI dessert) after 10–12 hours fasting and blood samples were collected before and 30, 60, 90, 120 min after consumption of the desserts. Afterwards, they were asked to evaluate the degree of preference, hunger and satiety as well as potential side effects of the desserts consumed.

Results: In the intervention group, HbA_{1c} ($p=0.015$), fasting blood glucose ($p=0.01$), body weight ($p<0.001$), body mass index (BMI) ($p<0.001$), waist circumference ($p<0.001$), hip circumference ($p<0.001$), blood pressure ($p<0.03$), SGPT ($p=0.02$) and γ GT ($p=0.04$) decreased significantly and there was a trend for a decrease in CRP ($p=0.09$) at 3 months in comparison with baseline. In the control group, there was a trend for decrease in HbA_{1c} ($p=0.08$), while weight, ($p<0.001$), BMI ($p<0.001$), waist ($p<0.001$) and hip ($p<0.001$) circumference and SGOT ($p=0.049$) decreased significantly at 3 months in comparison with baseline. At three months, the intervention group in comparison with the control group showed greater improvement in body weight ($p=0.05$), BMI ($p=0.032$), diastolic blood pressure ($p=0.03$), γ GT ($p=0.04$) and CRP ($p=0.02$). The area under the curve of blood glucose during the 2-hour period was significantly lower after consumption of the dessert of low GI/GL in comparison with the consumption of the common dessert ($p<0.001$). Moreover, the degree of preference and the feeling of hunger or satiety were not different after consumption of the two different desserts. Furthermore, 2.1% of the subjects who consumed the low GI/GL dessert and none of those who consumed the common dessert reported gastrointestinal side effects.

Conclusion: Consumption of desserts with low GI/GL in a balanced hypo-caloric diet has a positive impact on weight loss and improves metabolic parameters of patients with type 2 diabetes mellitus.

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A low-carbohydrate protein sparing modified fast diet compared with a low glycaemic index reduced calorie diet in obese type 2 diabetic patientsD. Vlachos¹, A. Ganotopoulou², C. Stathi³, A. Koutsovalis³, E. Diakoumopoulou³, D. Doulgerakis³, N. Tentolouris³, A. Melidonis², N. Katsilambros³¹Eurodiet Med, Athens, ²Diabetes Center, Tzanio General Hospital, Athens,³1st Department of Propaedeutic and Internal Medicine, Athens University Medical School, Laiko General Hospital, Greece.

Background and aims: Low-carbohydrate (LCD) and protein sparing modified fast diets (PSMF) seem intuitively attractive due to their potent antihyperglycemic effect while low-glycemic reduced calorie diets (LGID) are more common. This study aims at evaluating and comparing the effect of a LCD/PSMF diet with a LGID on the improvement of weight-related components, glycemic control and lipidemic profile in obese type2 diabetes patients.

Materials and methods: Seventy two type 2 diabetic patients [(32%) men, and (68%) women], 54.41±8.48 years old, BMI > 30 Kg/m² and type2 diabetes were randomized to either a LCD/PSMF ketogenic diet (800kcal/day progressively increasing to 1200kcal/day) or a low-glycemic index diet, reduced-calorie diet (500 kcal/day deficit from weight maintenance diet). All patients received group meetings, and exercise recommendation. Participants were encouraged to exercise for 30 minutes at least 5 times/week. At baseline and at all return visits participants recorded all of their current medications. Body weight, BMI, fat mass, fat-free mass, total cholesterol, LDL, HDL, triglycerides (TGs) and HbA1c were determined before and at 2, 9 and 22 weeks after the administration of each diet.

Results: Nine participants out of 40 and 8 out of 39 randomized to the LCD/PSMF and LGID group respectively, discontinued the study leaving 31 in the LCD/PSMF group and 32 in the LGID group for the analyses. Both interventions led to improvements in HbA1c, fasting glucose, fasting insulin and weight loss. At 22 weeks time interval the reduction of mean HbA1c was greater for the LCD/PSMF group (8.8 ± 1.8% to 7.3 ± 1.5%, p = 0.009) than for the LGID group (8.3 ± 1.9% to 7.8 ± 2.1% p= NS, between groups comparison p = 0.03). Reduction in fasting glucose was also greater in the LCD/PSMF (47.97±17.21 vs 24.13±12.21, p=0.021) than in the LGID group. Reduction of body weight was significant in the LCD/PSMF group (109.74±20.73 vs 93.81±19.13, p=0.001) but nonsignificant in the LGID group (103.50±14.84 vs 98.54±14.64, p=0.191). Waist circumference was significantly reduced in the LCD/PSMF group (131.28±12.70 vs 100.93±13.42, p<0.001) but not in the LGID group (126.66±9.34 vs 114.60 ±7.79, p=0.186). Only in the LCD/PSMF group there was a significant reduction in body fat mass (47.41±11.35 vs 34.70±10.40, p<0.001), in total cholesterol (p=0.001), in TGs (p=0.014), and in diastolic blood pressure (0.041) as well as a significant increase in HDL (0.036). All these improvements were statistically significant from the 9th week after the introduction of the LCD/PSMF diet. There was also a significant reduction in the insulin units in the LCD/PSMF group (60±32 vs 28±12 IU/day, p=0.022).

Conclusions: The LCD/PSMF diet compared to a LGID results: a) in a significantly greater weight loss, b) in a better glycemic control and lipidemic profile, c) greater reduction of BP and d) reduction and/or eliminate of medication.

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Specific nutrients, foods and risk of type 2 diabetes in subjects with IFG: the DR's EXTRA StudyH.M. Heikkilä^{1,2}, U. Schwab^{2,3}, B. Krachler¹, R. Kouki¹, R. Rauramaa^{1,4},¹Kuopio Research Institute of Exercise Medicine, ²School of Medicine, Clinical Nutrition, University of Eastern Finland, Kuopio Campus, ³Institute of Medicine, Kuopio University Hospital, ⁴Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Finland.

Background and aims: Insulin resistance and impaired insulin secretion, major pathophysiological mechanisms for Type 2 Diabetes (T2D), have both been found to be present in impaired fasting glucose (IFG). IFG precedes the development of T2D. Besides the effect of diet and physical fitness on insulin resistance, there are also findings of diet being linked to improved insulin secretion. However, currently only scarce data are available on the association of dietary factors and IFG. We therefore studied the association of selected food items and nutrients with IFG independent of cardiorespiratory fitness.

Materials and methods: The subjects were participants of our 4-year randomized controlled exercise and diet intervention study, The DR's EXTRA trial (ISRCTN45977199). We identified 126 subjects (84 men and 42 women) with IFG in our population based sample of 1261 individuals of 633 women and 628 men aged 57 - 79 years. IFG was defined as fasting plasma glucose 6.1-6.9 mmol/l and 2h plasma glucose < 7.8 mmol/l. Dietary intake was assessed by 4-day food records. Selected dietary factors for analysis were as follows: dietary fiber, saturated fat, polyunsaturated fat, cereal products, whole meal bread, fruits, berries, vegetables, butter, cheese, sausage, vegetable oil and sugar. Associations between dietary factors and glucose metabolism status were studied by logistic regression analysis. Data were adjusted for age and maximal cardiorespiratory fitness (VO₂max). Results are expressed as OR; 95 % CI. **Results:** Dietary fiber intake (g/1000kcal) was associated with decreased risk for IFG (OR 0.94; CI 0.90-0.99). The highest tertile of cereal products was also associated with decreased risk for IFG (OR 0.53; CI 0.32-0.87). Furthermore, the highest tertile of whole meal bread was associated with decreased risk for IFG (OR 0.48; CI 0.29-0.78). Saturated fat (E%) was associated with increased risk for IFG (OR 1.08; CI 1.01-1.14). Of the foods contributing to saturated fat intake sausage was associated with increased risk for IFG (OR 1.60; CI 1.06-2.41). Associations between the highest tertile of saturated fat and IFG (OR 1.51; CI 0.95-2.39) and the highest tertile of sausage and IFG (OR 1.48; CI 0.94-2.33) were of a borderline significance (P = 0.082 and 0.087, respectively).

Conclusion: Data suggest that dietary fiber, cereal products and whole grain products are protective against the risk of IFG, whereas saturated fat and foods contributing to saturated fat intake are associated with an increased risk of IFG.

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Menstruation dependent blood glucose variation in women with type 1 diabetes mellitusC. Kellner¹, N. Müller¹, C. Kloos¹, G. Wolf¹, J. Weiss², U.A. Müller¹;¹Department of Internal Medicine III, ²Department of Gynecology and Obstetrics, University Hospital Jena, Germany.

Background and aims: Some patients with type 1 diabetes mellitus experience a worsening in metabolic control at the beginning of menstruation. In a detailed German training programme for patients with type 1 diabetes a worsening in blood control with increased risk of ketoacidosis and hypoglycaemia was reported. Increased premenstrual blood glucose levels were discussed and the possible controversial reasons for this are speculative. This study was designed to investigate blood glucose levels and possible influencing factors e.g. pain, nutrition, activity, stress and mood dependent on menstruation.

Materials and methods: 20 menstrual cycles from women with type 1 diabetes mellitus (age 34y; diabetes duration 22y; HbA1c 7.2%, BMI 26kg/m², blood glucose self monitoring 5,9 tests/d, pump therapy 65%) were analysed by means of self monitored blood glucose, insulin dose and food intake. Physical activity and stress were documented using a 3 point Lickert-Scale and pain with a 10 point Lickert-Scale. The mood was measured with the Zersen mental-state-scale. The menstrual cycle can be divided into 3 sections: the menstruation period, with the first day of menstruation as the beginning, then the mid-cycle and thirdly the premenstrual period. Half the women took oral contraceptives. All patients had participated in a structured education in the last 10 years. Clinical and laboratory data were drawn from the electronic patient record. HbA1c was DCCT adjusted (normal mean 5.05%).

Results: In the premenstrual period mean blood glucose was 0.4 mmol/l higher compared to the menstrual period (8.0 vs. 7.6 mmol/l; p=0.04). Caloric intake increased by 8% (1888 kcal vs. 1722 Kcal/d; p=0.03). There was a slight increase in all nutrients: carbohydrates 197g vs. 189g (p=0.29), fat 17g vs. 11g (p=0.08) and protein 68g vs. 61g (p=0.015). No change in the food composition occurred during menstrual cycle. The mean insulin dose was 19 IU/d during the menstrual period, decreased to 18.3 IU/d mid-cycle-period (p=0.03 vs. menstrual period) and increased to 19.9 IU/d in the premenstrual period (p<0.001 vs. Mid-cycle). Pain (score max. 10) was scored higher during menstruation compared to the premenstrual period (1,5 to 0,4 p=0,005). There were no significant differences in documented physical activity, stress or mood. The increase of blood glucose (7.7 to 7.4mmol/l, p=0.35) and caloric intake (1829 vs. 1713 kcal/d, p=0.3) in the premenstrual phase was markedly less in women with oral contraceptives compared to women without (8.4 vs. 7.9 mmol/l, p=0.04; 1941 vs. 1731 kcal/d, p=0.063).

Conclusion: In women with type 1 diabetes we found a slight but significant premenstrual increase in blood glucose levels compared to the other sections of the cycle. This increase in blood glucose was not entirely compensated by the increased insulin dosage. The increase in blood glucose may be caused by increased food intake. No woman complained of premenstrual syndromes, pain, stress or reduction in physical activity. The small increase of blood glucose levels was clinically irrelevant.

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A diet high in whole grain, fatty fish, and bilberry improves markers of endothelial function and inflammation in individuals with impaired glucose metabolism

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Background and aims: Low-grade inflammation and endothelial dysfunction may play a role in the pathogenesis of the metabolic syndrome (MetS), type 2 diabetes mellitus and cardiovascular diseases. We sought to evaluate if a diet high in fatty fish, bilberry, and whole grain products including breads with a low insulin response (“HealthyDiet”) improves biomarkers reflecting inflammation and endothelial dysfunction in individuals with impaired glucose metabolism and features of the MetS.

Materials and methods: Altogether 104 participants (59 ± 7 yrs; BMI: 31.1 ± 3.5 kg/m²) with impaired glucose metabolism (IFG or IGT) and features of the MetS who were randomized to either the “HealthyDiet” (n=36), a Whole Grain-Enriched Diet (WGED, n=34) or a control diet (n=34) for 12 weeks were analyzed. Measurements were taken at baseline and at week 12. Serum hsCRP (high-sensitivity C-reactive protein) concentration was determined by nephelometry and E-selectin concentration by ELISA.

Results: Serum E-selectin decreased in the “HealthyDiet” group in all participants (33.2 ± 13.8 vs. 31.5 ± 13.3 µg/L, % change: -4.2 (-10.1; 1.7); p=0.04) and also after excluding those using statins (n=27) (35.1 ± 15.2 vs. 32.7 ± 14.7 µg/L, % change: -6.7 (-11.5; 1.9); p=0.004). The change in E-selectin was significantly different from the change in the control group (p=0.02). Serum E-selectin did not change significantly in the WGED or in the control groups either in all participants (31.7 ± 10.7 vs. 32.3 ± 12.4 µg/L and 28.5 ± 11.5 vs. 30.0 ± 12.7 µg/L, respectively; p>0.05 for both) or after excluding those using statins (31.7 ± 10.8 vs. 31.7 ± 12.3 µg/L and 30.5 ± 11.8 vs. 32.2 ± 13.0 µg/L, respectively; p>0.05 for both). Serum hsCRP levels decreased in both the “HealthyDiet” (1.9 [0.8; 3.6] vs. 1.1 [0.8; 2.6] mg/L, % change: -16.8 (-40.7; 0.00) p=0.04) and WGED (2.1 (0.7; 4.1) vs. 1.0 (0.6; 2.3) mg/L, % change: -27.2 (-58.6; 0.00); p=0.006) groups in participants not using statins (n=24 and n=25, respectively). Serum hsCRP did not change in the control group (p>0.10) and was significantly different from the change in the WGED group (p=0.03). The increase in the intake of long-chain n-3 fatty acids and fiber during the study were both associated with the decrease in serum E-selectin (β=-0.30 and β=-0.26, p<0.05 for both). The amount of bread with a low insulin response consumed during both the “HealthyDiet” and WGED interventions was associated with the decrease in hsCRP levels (β=-0.51, p<0.001).

Conclusion: Our results suggest that the combined effect of dietary whole grain, fatty fish and bilberries may have a benefit on endothelial dysfunction and inflammation in individuals with impaired glucose metabolism and features of the MetS.

Clinical Trial Registration Number: NCT00573781

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Dietary fibre and glycaemic control in patients with diabetes: a systematic review and meta-analysis of randomised clinical trials

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Background and aims: Increased fiber intake has been associated with improved glycemic control in patients with diabetes and represents a useful dietary tool to reach glycemic targets. The aim of this study was to assess the effect of fiber intake on glycemic control of patients with diabetes by a systematic review and meta-analysis of randomized controlled trials (RCT).

Material and methods: To identify RCTs that report the effect of fibers on HbA1c and fasting plasma glucose (FPG) (outcomes) the Pubmed, Embase, and Scopus databases were searched up to January, 2011. Reference lists of retrieved articles were also checked. Search strategy included medical subject headings related to diabetes, fiber, and RCT. Study selection and data extraction were independently performed by two investigators. Studies were considered eligible for inclusion if they fulfilled the following criteria: evaluated the effect of fiber diet on HbA1c and/or FPG of adults patients with type 1 or type 2 diabetes, had at least eight-weeks duration, and described mean (or differences between means) and standard deviations of outcomes at baseline and end-of-intervention. Fiber data were described as the difference between intervention and control diets. Changes in HbA1c (percentage) and FPG (absolute values) were reported as difference between arithmetic mean before and after intervention. Pooled estimates of weighted mean differences (WMD) and 95% CIs were obtained by random-effects models. The Cochran's x test was used to evaluate heterogeneity between studies and I² test to evaluate the heterogeneity magnitude. Publication bias was assessed by Begg's and Egger's tests. Statistical analyses used Stata 11.0 software.

Results: From 18,446 studies initially identified, 60 were selected for full text evaluation based on title, abstract, and reference lists. Thereafter, 12 studies (including 14 reports) with eight to 24 weeks duration (nine with parallel and three with crossover designs) fulfilled the inclusion criteria providing data from 659 diabetic patients, aged 58.2 years (28 to 69.1), 61.3% males, and 91.7% with type 2 diabetes. In five reports the intervention was diet with foods rich in fiber (fiber difference=3.0 to 22.5 g/day). In seven reports the intervention was dietary fiber supplement (fiber difference=3.5 to 16.5 g/day): goma-guar (4 reports), psyllium (2 reports), or β-glucan (1 report). HbA1c change did not differ between intervention and control diets (14 reports; WMD = -1.37% [-4.08, 1.34]; I²=80.3%, P<0.001). Pooled data about the effect of high fiber intake on FPG (9 reports) also revealed no difference as compared to control diets (WMD = -4.28 mg/dl [-15.09, 8.57]; I²=93.6%, P<0.001). These results did not change when sensitivity analyses were performed considering diets with foods rich in fiber or with fiber supplements. There was no evidence of publication bias, both for HbA1c and FPG (Funnel plots and the Egger regression test; P>0.10).

Conclusions: The present systematic review and meta-analyses did not support a beneficial effect of an increased fiber intake on glycemic control in patients with diabetes. However, studies are still necessary to evaluate this topic, since a high heterogeneity between RCTs was demonstrated.

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Fibres intake is associated with lower inflammation among type 1 diabetes subjects

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Background and aims: Epidemiological studies have shown that vegetables intake is associated with a lower risk of cardiovascular events, possibly due to anti-inflammatory effects. In fact, lower C-reactive protein (CRP) levels were associated with high dietary intake of fruits, vegetables and grains - fiber sources foods. Patients with diabetes display a state of chronic low-grade inflammation could be related to chronic microvascular complications, and its association with insulin resistance and metabolic syndrome. In this way, the aim of this study was to evaluate an association between high-sensitivity (hs) CRP levels and composition of the usual diet of patients with type 1 diabetes

mellitus (T1DM), especially the association between the CRP-US levels and the consumption of fibers.

Materials and methods: Cross-sectional study with T1DM outpatients from the Endocrine Division of the HCPA was performed. Patients underwent clinical assessment (blood pressure levels, assessment of microvascular complications, and anthropometric measurements), nutrition (weighed diet records in two nonconsecutive week-days and one day-off, with concomitant urinary collection 24h to measurement of urinary urea) and laboratory [glycemic control, lipids, renal function (serum creatinine and microalbuminuria), and high-sensitivity CRP (hs-CRP) [immunoturbidimetric method (Siemens Advia 1800 Clinical Chemistry System, Germany)]. Patients with hs-CRP values higher than 10 mg/dl were excluded.

Results: There were 84 patients with T1DM with 41.6 ± 9.8 years old, 18 ± 9 years of diabetes duration, 50.6% male and 79.3% whites. The BMI was 24.6 ± 3.4 kg/m², waist circumference = 83.4 ± 9.3 cm, fasting glucose = 211 ± 128 mg/dl, HbA_{1c} = $9.3 \pm 2\%$, total cholesterol = 190 ± 35 mg/dl, HDL = 60.8 ± 15.8 mg/dl, triglycerides = 86 (68–118) mg/dl, urinary albumin excretion = $6.2\mu\text{g}/\text{min}$ (0.0–16), serum creatinine = 0.8 mg/dl (0.5–2.0) and values of hs-CRP = 1.67 mg/dl (0.7 to 4.71). The analysis of daily nutrient intake (% of total energy) showed carbohydrate intake = $48.9 \pm 7.8\%$, protein = $17.9 \pm 3.6\%$, total lipids = $33.3 \pm 8.6\%$, saturated fatty acids (FA) = $10.1 \pm 2.9\%$, monounsaturated FA = $11.6 \pm 3.8\%$, polyunsaturated FA = $8.1 \pm 4.8\%$, trans-FA = $0.5 \pm 0.4\%$, and cholesterol 226 ± 115 mg. Daily intake of total fiber was 23.1 ± 8.7 g. The total fiber intake was negatively correlated with the hs-CRP values: $r = 0.30$ ($P = 0.01$) and this association was supported on multiple linear regression model ($R^2 = 0.21$, $\beta = -0.58$, $P = 0.02$), adjusted for HbA_{1c}, BMI, and diastolic blood pressure (variables come from the association on univariate analysis). To facilitate understanding hs-CRP levels and fibers were stratified to each 0.5 mg/dl and portions of 5g, respectively. In an ordinal regression model, categories of hs-CRP (between 0–0.5 mg/dl; 0.51–1 mg/dl, 1–1.5 mg/dl, 1.51–2 mg/dl and ≥ 2.1 mg/dl) were included as dependent variable and the fiber intake levels, BMI and HbA_{1c} were included as independent variables. Each 5g of additional intake fiber higher than 15g/day reduced 0.5 mg/dl on hs-CRP levels.

Conclusion: The intake of at least 20g/day of fiber is associated with a reduction on hs-CRP levels in patients with T1DM.

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demonstrated a reduction from D-0 ($15.7 \pm 1.2\text{g}$) to D-30 ($9.1 \pm 0.5\text{g}$) ($p < 0.001$). In groups A and B there was no significant difference in FBG or 2hr post prandial BG from D-0 to D-30, in diabetes-induced animals group D demonstrated a significantly lower FBG on D-20 and D-30, and 2hr post prandial BG was also significantly lower on D-30. In CE administered groups total and LDL cholesterol levels were lower on D30 compared to D0 in healthy and diabetes-induced animals ($p < 0.001$). HbA_{1c} of group-C increased from D-0 ($2.2 \pm 0.1\%$) to D-30 ($4.5 \pm 0.9\%$) ($p < 0.001$), in group-D it remained unchanged.

Conclusion: Cinnamomum zeylanicum lowered blood glucose, reduced food intake and improved lipid parameters. Its efficacy as a standalone therapy and impact on macro and microvascular complications requires further study.

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Effects of cinnamomum zeylanicum (Ceylon cinnamon) on blood glucose and lipids in Sprague-Dawley rats

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Background and aims: Diabetes is a leading cause of morbidity and mortality in the world. Recent studies have demonstrated the beneficial effects of herbal remedies in its treatment. We evaluated short and long term effects of Cinnamomum zeylanicum on food consumption, body weight, glycaemic control and lipids in healthy and diabetes-induced rats

Materials and methods: The study was conducted in two phases, using Sprague-Dawley rats (body weight- $190 \pm 25\text{g}$, age 3–4 months) as the animal model. Phase-I evaluated short term effects of cinnamon on blood glucose (BG) in fasting (Group-1 and 2) and following a standard oral glucose (OG) load (Group-3 and 4), using 36 healthy rats in four equal groups. Group 1 and 3 received distilled-water (DW) and groups 2 and 4 received cinnamon-extracts (CE). Serial BG values were measured from 0–24hrs. DW/CE was administered at 0-hrs in group 1 and 2 and 0.5-hrs in group 3 and 4 (standard OG load given at 0-hrs). Phase-II evaluated long term effects of cinnamon on food consumption, body weight, BG and lipids over one month. Group-A ($n=8$, DW) and Group-B ($n=8$, CE) were healthy rats, while Group-C ($n=5$, DW) and Group-D ($n=5$, CE) were diabetes-induced rats. Serum total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and HbA_{1c} was measured on D-0 and D-30. FBG, 2 hour post-prandial BG and body weight were measured on every 5th day, together with daily food consumption.

Results: Phase I-There was no significant difference in serial BG values of groups 1 and 2. Mean percentage reduction of BG from FBG at each hour was greater in group-2 at 0.5, 1 and 2-hrs ($p > 0.05$). Group-4 demonstrated a faster decline in BG following OG load in comparison to group-3 ($p < 0.05$). Phase II-There was no difference in food consumption of healthy rats (Group-A and B) between D-0 and D-30, in diabetes-induced rats group-D

PS 074 Fat in the diet

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Genetically determined metabolic responses to isocaloric high carbohydrate and high saturated fat diets in healthy twins

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Background and aims: Diets high in carbohydrates and/ or fat can induce adiposity and diabetes mellitus type 2. Studies in humans showed rapid development of insulin resistance when applying a hypercaloric high fat diet. This study investigated the effects of a sudden switch from healthy low fat to isocaloric high fat diet on body weight (BW), insulin sensitivity (S_i), serum lipids and inflammatory cytokines in healthy twins.

Materials and methods: 52 monozygotic and 8 dizygotic twins (BMI 18–35 kg/m², twin BMI-difference ≤ 3 kg/m², age 18–70) were investigated for 12 weeks. An isocaloric diet rich in carbohydrates (55% carbohydrates, 15% protein, 30% fat) was applied with dietary counselling for 5 weeks and afterwards nutrients were supplied for 6 days. Then an isocaloric diet rich in saturated fat (40% carbohydrates, 15% protein, 45% fat) was applied for 6 days with nutrients supplied, followed by 4 weeks with dietary counselling and for another 6 days when nutrients were supplied again. An intravenous glucose tolerance test (IVGTT), anthropometry and blood tests for total Cholesterol, non esterified fatty acids (NEFA) and inflammatory cytokines were performed after the period of diet rich in carbohydrates (Carb), after the first 6 days (HF_{short}) and at the end of the period of diet rich in fat (HF_{long}). The IVGTT was analyzed according to the Minimal Model. Data represents the mean \pm SEM. One-way ANOVA with Bonferroni posttest was used to test the difference between the means of the groups. Pearson's correlation coefficient (PCC) was used to test the correlation of parameters within the pairs of twins.

Results: Mean BW was constant over the entire period of intervention: Carb 69.06 \pm 1.79 kg, HF_{short} 69.04 \pm 1.77 kg, HF_{long} 69.34 \pm 1.80 kg. Total cholesterol levels showed a moderate, but insignificant, increase from Carb (4.60 \pm 0.12 mmol/l) over HF_{short} (4.75 \pm 0.12 mmol/l) up to HF_{long} (4.92 \pm 0.12 mmol/l), however were always highly correlated within the pairs of twins: PCC: Carb: 0.781, HF_{short}: 0.705, HF_{long}: 0.844. Interestingly, NEFA significantly decreased at the end of the period of diet rich in fat compared to the end of the period of diet rich in carbohydrates: 0.60 \pm 0.03 mmol/l, HF_{short} 0.52 \pm 0.03 mmol/l, HF_{long} 0.49 \pm 0.03 mmol/l ($P < 0.05$ HF_{long} vs. Carb) and were only correlated within the pairs of twins after HF_{short} (PCC: 0.716). Insulin sensitivity (S_i) did not change: Carb: 13.14 \pm 1.52 [(mu/l)⁻¹.min⁻¹], HF_{short}: 15.09 \pm 3.07 [(mu/l)⁻¹.min⁻¹], HF_{long}: 12.74 \pm 1.33 [(mu/l)⁻¹.min⁻¹] and was only correlated within the pairs of twins after Carb (PCC: 0.458). MCP-1 as a marker of inflammation decreased moderately, but not significantly, over the time of intervention (Carb: 335.6 \pm 13.4 pg/ml, HF_{short}: 324.1 \pm 10.8 pg/ml, HF_{long}: 311.4 \pm 11.6 pg/ml) and was always significantly correlated within the pairs of twins: PCC: Carb: 0.491, HF_{short}: 0.702, HF_{long}: 0.607.

Conclusion: These results demonstrate the degree of genetic determination of metabolic and inflammatory responses to isocaloric dietary interventions. Supported by: BMBF

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A high fat diet improves glycaemic control compared with low fat diet: a 24-month randomised prospective study of patients with type 2 diabetes in primary health care

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Background and aims: Little is known about the long-term effects of a low carbohydrate/high fat diet in type 2 diabetes. We aimed to prospectively explore the efficacy of traditional low-fat diet compared with a high-fat dietary recommendation on the metabolic profile in patients with type 2 diabetes.

Materials and methods: We randomized 61 patients (allocation 1:1) with type 2 diabetes to a low-fat diet (55–60 E% from carbohydrates, LFD) or a diet with maximal intake of 20E % from carbohydrates and 50E% from fat (HFD) in order to lose weight at two primary health care centers in Sweden. Both groups were asked to limit the total caloric intake to 1600 kcal for women/day and 1800 kcal/day for men. The patients were given dietary advises on 4 occasions in group sessions, and had access to the same dedicated dietician who also provided patients with suitable cooking recipes. The patients were followed for two years.

Results: No subjects were lost to follow up, weight loss was similar in both groups and maximal at the 6-month control (reduction at 6 months, LFD: -3.99 \pm 4.1 kg, HFD: -4.31 \pm 3.6 kg, $p=0.8$ for comparison between groups, at 12 months: LFD: -3.21 \pm 4.2 kg, HFD: -3.15 \pm 4.0 kg, $p=0.96$ between groups and at 24 months: LFD: -2.84 \pm 4.9 kg, HFD: -2.34 \pm 5.1 kg, $p=0.7$ between groups). Based on 3-day food reports, subjects randomized to HFD reduced carbohydrates and increased energy intake from fat compared with the contrasting group from baseline compared with 6 months (change in E% from fat +12.6 \pm 8.1%, from carbohydrates -17.2 \pm 8.7 E%, corresponding values for the LFD group: fat -0.93 \pm 8.4 E% from carbohydrates -0.11 \pm 8.2%, p for comparison of changes in between groups < 0.0001 for both fat and carbohydrates). While LDL-cholesterol remained unchanged in both groups at the 6 and 12 month controls, HDL cholesterol was increased at 6 months in the HFD (from 1.13 \pm 0.33 mmol/l to 1.25 \pm 0.47, $p=0.02$) and in both groups at 12 months (HFD from 1.13 \pm 0.33 mmol/l to 1.24 \pm 0.38, $p=0.02$ and in LFD from 1.09 \pm 0.29 mmol/l to 1.17 \pm 0.24, $p=0.004$). HbA1C was lowered in the HFD at the 6 month control ($p=0.004$) and tended ($p=0.12$) to be lower also at the 12 month control but not at the 24 month control ($p=0.9$). HFD baseline: 6.61 \pm 0.97%, 6-month: 6.15 \pm 1.0%, 12 month: 6.40 \pm 1.2%, 24 month: 6.62 \pm 2.7% corresponding values for the LFD group: 6.34 \pm 0.78%, 6-month: 6.25 \pm 0.93%, 12 month: 6.41 \pm 1.1%, 24 month: 6.50 \pm 1.0%. The change in HbA1C from baseline to the 6-month control was statistically significant between the groups after correction for the concomitant reduction in medication ($p=0.017$ – 0.025 depending on the correction parameters).

Conclusion: HFD can be more effective to achieve glycemic control after 6-months than LFD at similar weight loss when reduction of medication is taken into consideration. Since no adverse changes occurred regarding blood lipids by the HFD, this diet seems safe as a life style intervention to reduce the need for medication in type 2 diabetes. Furthermore, we demonstrated that improvement of glycemic control or body weight can be achieved with the input of very modest amount of resources by the health care provider, in particular if a HFD is recommended.

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Impact of PUFA-omega 3 on body composition and adipocytokines levels in metabolic syndrome patients

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Background and aims: The aim of this study was to assess the impact of 18 months administration of ω -3 PUFA supplements on body composition and adipocytokines levels in metabolic syndrome patients.

Materials and methods: In the study were included 323 patients, 164 women and 159 men, aged 63 \pm 7.3 years, with metabolic syndrome. They were allocated to 2 groups, matched by sex, age and weight: group A - received a nutritional program consisting in diet intervention and regular physical activity; group B - received the same nutritional program + capsules of fish oil (1g eicosapentanoic acid, 1g docosahexanoic acid, 0,1g α -tocopherol acetate). Body fat mass (BFM) and body fat percent (%BF) were measured using bioimpedance analysis. Adipocytokines levels were assessed according to standard procedures. The oxidative stress was assessed using FormOX systems monitor on a blood drop. Patients were evaluated before and after the intervention.

Results: Baseline characteristics were similar between groups. After 18 months, omega-3 supplements determined a significant improvement of adipocytokines levels and body composition in group B compared with group A: leptin - 14 \pm 2.8 vs. 17 \pm 3.9; adiponectin - 12.25 \pm 3.3 vs. 9.88 \pm 2.7; leptin/adiponectin ratio - 1.14 \pm 0.84 vs. 1.72 \pm 1.44; body fat mass (BFM) - 24.82 \pm 3.4 vs. 28.67 \pm 5.5; body fat percent (BF%) - 25.92 \pm 1.6 vs. 29.64 \pm 4.8; waist-to-hip ratio - 0.98 \pm 0.06 vs. 1.06 \pm 0.05. BFM was statistically correlated with leptin values ($p < 0.0001$), adiponectin values ($p < 0.001$) and oxidative stress ($p < 0.001$), while %BF was correlated with leptin values ($p < 0.0001$), leptin to adiponectin ratio ($p < 0.0001$) and oxidative stress ($p < 0.002$).

Conclusion: Our study concluded that in metabolic syndrome patients, PUFA-omega 3 enriched diets may decrease the oxidative stress and improve adipocytokines levels and body composition.

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Dietary n-3 fatty acids supplied as phospholipids are more effective than triglycerides in reducing obesity-associated disorders: evidence for a link with the endocannabinoid system

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Background and aims: The endocannabinoid system is a significant player in the control of energy balance and is overactive in obesity. Dietary n-3 polyunsaturated fatty acids (n-3 PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), could prevent development of obesity and insulin resistance. We studied metabolic effects of n-3 PUFA in the diet either in the form of phospholipids or triglycerides.

Materials and methods: Three-month-old C57BL/6J male mice were fed for 4 months a corn oil-based high-fat diet (cHF; lipids ~35% wt/wt) to induce obesity. Mice were then randomly divided in three subgroups: 1) cHF diet supplemented with metformin (2 g/kg diet; “cHF+M” diet - control); 2) cHF+M diet, in which part of lipids was replaced by a high-DHA n-3 PUFA concentrate (EPAX 1050 TG; 60% EPA+DHA wt/wt; “E” diet); and 3) cHF+M diet, in which part of lipids was replaced by a novel marine phospholipid concentrate containing mainly DHA (G3; 27% EPA+DHA wt/wt; “G3” diet). Experimental diets were matched for the total EPA and DHA content (31.5 mg/g diet) and were administered for 9 weeks. Markers of glucose and lipid homeostasis, hepatic steatosis, adipocyte hypertrophy, and endocannabinoid levels were assessed.

Results: Both E and G3 diets tended to lower weight gain while reducing the weight of abdominal fat depot (cHF+M, 2440 ± 206; E, 1906 ± 231; G3, 1978 ± 164 mg; p=0.021 and p=0.027 for cHF+M vs. E and G3, respectively). Plasma levels of non-esterified fatty acids and total cholesterol were similarly reduced by both treatments, while G3 exerted stronger effects on plasma triacylglycerols (cHF+M, 1.09 ± 0.08; E, 0.78 ± 0.12; G3, 0.66 ± 0.04 mmol/l; p=0.016 and p<0.01 for cHF+M vs. E and G3, respectively). Only G3 reduced plasma insulin (cHF+M, 1.78 ± 0.32; E, 1.29 ± 0.30; G3, 0.91 ± 0.11 ng/ml; p=0.034 for cHF+M vs. G3) and HOMA-IR (cHF+M, 17.7 ± 3.6; E, 9.9 ± 2.0; G3, 7.3 ± 1.0; p=0.02 for cHF+M vs. G3). Furthermore, hepatic steatosis (cHF+M, 160 ± 17; E, 83 ± 15; G3, 41 ± 4 mg/g tissue; p<0.001 for cHF+M vs. G3) and adipocyte hypertrophy (cHF+M, 5961 ± 381; E, 5195 ± 349; G3, 4433 ± 127 μm²; p=0.013 for cHF+M vs. G3) were both more effectively reduced by G3. These effects were associated with reduced levels of 2-arachidonoylglycerol (2-AG; cHF+M, 187 ± 36; E, 79 ± 13; G3, 46 ± 4 ng/g tissue; p<0.001 for cHF+M vs. G3) and elevated levels of N-docosahexaenoyl ethanolamine (DHEA; cHF+M, 2.40 ± 0.15; E, 14.92 ± 1.63; G3, 31.37 ± 7.26 ng/g tissue; p<0.001 for all comparisons) in adipose tissue.

Conclusion: Compared with marine triglycerides, dietary n-3 PUFA as phospholipids ameliorated more efficiently metabolic defects such as hypertriglyceridemia, adipocyte hypertrophy, and hepatic steatosis. Possible mechanisms for this differential effect could include more efficacious reduction of endocannabinoid 2-AG and increased production of anti-inflammatory endocannabinoid-like molecule DHEA.

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Effect of an alpha-linolenic enriched diet on markers of endothelial function, inflammation and glucose metabolism in patients with metabolic syndrome during weight reduction

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Background and aims: The metabolic syndrome is associated with visceral obesity and closely linked to a proinflammatory state and endothelial dysfunction. Improvement of inflammation and endothelial function by marine n-3 fatty acids has been widely evaluated, whereas the effects of the plant-based n-3 fatty acid alpha-linolenic acid (ALA) are largely unknown. In a controlled dietary study, we investigated the effects of ALA on endothelial function, inflammation and glucose metabolism in patients with metabolic syndrome (MBS) during a six month weight reduction program with natural food diets.

Material and methods: 81 patients (age: 51.3±10.2 years; body weight: 98.4±18.0 kg, without intergroup difference at baseline) with diagnosed MBS according to the criteria of the International Diabetes Federation were enrolled in the study with a parallel design after stratification according to gender, age and manifestation of diabetes. During the six month dietary intervention, all patients received an energy-reduced diet which had a moderate carbohydrate (41% of energy) and fat (38% of energy) content, but a low content of saturated fatty acids (< 10% of energy). In the intervention group (IG), the diet was enriched with rapeseed oil, leading to an ALA intake of 3.6 g/d, while the control group (CG) achieved an olive oil-rich diet (ALA intake: 0.8 g/d).

Results: After six months, both groups showed a similar weight reduction (-7.8 kg in the IG vs -6.0 kg in the CG) and decline in body fat (-5.8 kg vs -4.2 kg). The concentrations of ADMA and YKL-40 significantly decreased in the IG (-5.5%; p<0.01 and -24.8%; p<0.001) but not in the CG (-4.0%, p=0.154; -12.2%, p=0.152) with intergroup differences for YKL-40 (p=0.018 for time x group), indicating an improvement in endothelial function due to ALA enrichment. The values of sE-Selectin significantly decreased in both groups (-20.7% for IG, p<0.001; -10.8% for CG, p<0.001) without significant intergroup differences (p=0.063 for time x group). The inflammatory markers hsCRP and TNF-α were also significantly reduced in the IG and CG (p<0.05 for both groups and parameters), while the change of hsIL-6 was detected in IG only (-12.8%, p<0.05) without differences between groups (p for time x group: hsTNF-α p=0.794, hsCRP p=0.279, hsIL-6 p=0.838). With regard to the glucose metabolism, we observed a slight decrease in serum glucose levels, accompanied by a significant reduction in insulin (p<0.001 for both groups) and intact proinsulin (IG p<0.001; CG p<0.05). In addition, systolic and diastolic blood pressure were reduced in both groups, but the decrease in the latter was significantly more pronounced in the IG as compared to the control group (IG -8.4 mmHg vs CG -4.4 mmHg; p=0.026 for time x group).

Conclusion: An ALA-enriched fat-moderate weight reduction diet led to a more pronounced improvement of the metabolic risk profile in patients with metabolic syndrome than did a similar diet with a low content of ALA. Especially, two recently established important markers of endothelial function, YKL-40 and ADMA, were favorably influenced by the plant-based n-3 fatty acid alpha-linolenic acid.

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The effect of vegetarian diet on fatty acid composition of serum phospholipids and the association with insulin sensitivity and visceral fat in subjects with type 2 diabetes

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Background and aims: Insulin sensitivity is related to the composition of fatty acids in the serum and tissue lipids. The aim of our study was to examine the effect of vegetarian vs. conventional diet on fatty acid composition of serum phospholipids and to test the relations to insulin sensitivity and visceral fat content in subjects with T2D.

Materials and methods: Subjects with T2D (n=74) were randomly assigned to the experimental group (EG, n=37) following vegetarian diet or the control

group (CG, n=37) following conventional diet. Both diets were calorie-restricted (~500 kcal/day). Participants were examined at baseline, 12 weeks of diet intervention and 24 weeks (subsequent 12 weeks of diet were combined with aerobic exercise 3 hours/week). The fatty acid composition of serum phospholipids was measured by gas liquid chromatography. Insulin sensitivity was measured by the hyperinsulinemic (1 mU.kg⁻¹.min⁻¹) isoglycemic clamp. Visceral fat was measured by magnetic resonance imaging.

Results: The linoleic acid (18:2n6) increased in EG (p=0.04) while it decreased in CG (p=0.04) in response to dietary interventions (group x time p<0.001). It did not change significantly after the addition of exercise in either group. The highly unsaturated n6 fatty acids decreased in both groups from week 0 to 24 (p<0.001 for EG; p=0.03 for CG), more in EG (group x time p=0.01), especially due to decrease of arachidonic acid (20:4 n6) in both groups (p<0.001 for EG; p=0.05 for CG), that was accordingly greater in EG (group x time p=0.01). Metabolic clearance rate of glucose (MCR) increased in both groups from week 0 to 24 (p<0.001 for each group), more in EG (group x time p=0.04). Volume of visceral fat decreased in both groups from week 0 to 24 (p<0.001 for each group), more in EG (group x time p=0.007). In EG, changes in the linoleic acid (18:2 n6) correlated positively with changes in MCR (r=+0.22; p=0.04) and negatively with changes in volume of visceral fat (r=-0.28; p=0.05).

Conclusion: Vegetarian diet increased the content of linoleic acid in serum phospholipids. Its changes were associated with changes in MCR and visceral fat. The results support the hypothesis that these changes in linoleic acid could be involved in greater capacity of calorie-restricted vegetarian diet to improve insulin sensitivity compared to conventional diabetic diet.

Clinical Trial Registration Number: NCT00883038

Supported by: IGA MZCR NS/10534-3

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Effects of daidzein and genistein on glycaemic control and lipid metabolism in Chinese women with prediabetes or untreated early diabetes: a 6-mo double-blind, randomised, placebo-controlled trial

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Background and aims: Evidence from observational, in vitro, animal and some small sample human studies have suggested that soy isoflavones have favorable effects on glucose and insulin, but intervention studies especially the purify isoflavones studies in humans are limited, and the results are controversial. We conduct this study to evaluate the effect of purify genistein and daidzein on glycemic control and lipid metabolism in adult women with impaired glucose regulation.

Methods: This randomized, double-blind, placebo-controlled study included 165 Chinese adult women age from 30 to 70 years with prediabetes or early untreated diabetes. Participants were randomly assigned to one of three arms with a daily intervention of 10 g soy protein and none (control group), 50 mg daidzein (daidzein group), and 50 mg genistein (genistein group) for 24 weeks. Blood was collected on baseline, the 12weeks, 24 weeks for the measurements of fasting glucose, HbA1C, lipids (total, HDL, and LDL cholesterol, and triglycerides) lipoprotein (a) and high sensitive C create protein (hsCRP), and glucose concentrations at 30, 60, 120, 180 minutes post 75g oral glucose load.

Results: 157 and 152 subjects completed the follow up measures at 12 and 24 weeks, respectively. Twelve weeks or 24 weeks treatment with purified 50mg genistein or 50mg daidzein did not result in any significant difference in the changes of fasting glucose and HbA1C, glucose concentrations at 30, 60, 120, 180 minutes post 75g oral glucose load, and the area under the curve of glucose among the three treatment groups (all p>0.05). There was no significant difference in the changes in total, HDL and LDL cholesterol and triglycerides, lipoprotein (a) and hsCRP, microalbuminuria and Microalbuminuria / urine creatinine in the three arms (all p>0.05), the main outcome of 24weeks treatment are display in the table.

Conclusion: Our findings suggest neither purify genistein nor daidzein has a significant effect on glycaemic control and lipid metabolism in Chinese women with prediabetes and diet-controlled type 2 diabetes.

The changes of main outcomes after treatment of 24 weeks* (mean±SD)

	Placebo group (n=47)	Daizein group(n=51)	Genistein group(n=54)	P-value (ANOVA)
Fasting glucose(mmol/L)	-0.135±0.884	-0.105±0.813	-0.330±0.787	0.325
120-mins postload glucose(mmol/L)	-0.896±2.500	-0.377±2.303	-0.864±2.475	0.494
AUC of glucose	-136±304	-63±288	-123±314	0.462
HbA1c(%)	-0.156±0.775	-0.090±0.400	0.070±0.716	0.203
TC(mmol/L)	-0.198±1.049	-0.070±0.648	-0.118±0.961	0.782
TG(mmol/L)	-0.031±0.713	-0.042±0.857	-0.112±0.612	0.831
HDL(mmol/L)	0.013±0.852	0.002±0.219	0.014±0.222	0.963
LDL(mmol/L)	-0.126±0.852	-0.102±0.671	0.016±0.672	0.578
HsCRP(mg/L)	-0.151±1.837	-0.236±1.528	-0.864±2.482	0.184
Microalbuminuria / urine creatinine	0.001±0.018	0.003±0.015	0.006±0.055	0.767

* Values of change were calculated as [follow-up value (24 weeks) - baseline value (0 week)]

Clinical Trial Registration Number: NCT 00951912

Supported by: DHGDP(No: A2008158); CSN; Danone Institute

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Mixed carotenes reduce oxidised LDL in human volunteers

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Background and aims: Increases in oxidized low-density lipoproteins (oxLDL) contribute to the development of atherosclerosis. A 4-armed randomized, controlled trial was performed in 60 adult participants who consumed fewer than 3.5 servings of fruits and vegetables per day in order to test the hypothesis that a mixed carotene matrix would reduce biomarkers of cardiovascular risk and post-prandial oxidative stress.

Materials and methods: We randomized participants to 28-days of supplementation with either a safflower oil placebo; small, organic vegetable-based soups and/or salads designed to be rich in pre-specified mixed carotene fractions (provided to the participants); or one of two dietary supplements containing natural, mixed carotenes. Participants presented to the Clinical Research Center in the University of Washington Medical Center at the beginning and end of the trial in order to consume a standardized control meal. Blood samples were collected to measure changes in fasting cardiovascular risk factors and post-prandial oxidized low-density lipoprotein (pp-oxLDL). Outcome measures were evaluated between groups by ANOVA and within group by two-sided, paired t-tests.

Results: Forty-seven participants completed the trial. Total carotene serum status increased significantly in two groups (P≤0.01 for each within group change; P<0.0001 by ANOVA), and select carotene fractions including alpha- and beta-carotene and lutein increased in all three active groups (P≤0.05 for within group change). Significant reductions in LDL-C were measured in the two dietary supplement groups including Group B: -8.0 mg/dl, 95% CI: -3.7 to -12.3 mg/dl, P=0.002 for change; and Group C: -8.8 mg/dl, 95% CI: -1.5 to -16.1 mg/dl, P=0.02 for change (P=0.002 by ANOVA). Change in 1-hour pp-oxLDL concentration differed significantly between groups (P=0.03 by ANOVA) and reductions were measured in fasting, 1, 2 and 4-hr pp-oxLDL in both supplement groups (P<0.05 for each).

Conclusions: The results of this clinical trial suggest a mixed, carotene matrix may impact cardiovascular risk by lowering LDL-C and pp-oxLDLs.

Clinical Trial Registration Number: 01175577

Supported by: 1KL2RR025015-01

PS 075 The three G's: Glucokinase, Glucagon receptors and G-protein coupled receptors

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Pharmacokinetics, pharmacodynamics and tolerability of the glucokinase activator AZD1656, after single ascending doses in healthy subjects

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Background and aims: AZD1656 is a glucokinase activator with a postulated dual mechanism of action by activating glucokinase in the liver and pancreas and with the potential to deliver effective glucose-lowering in T2DM. Two single blind, randomized, placebo-controlled studies evaluated the pharmacokinetics and pharmacodynamics of AZD1656 in Western (SAD) and Japanese (JSAD) healthy male subjects after oral single ascending doses.

Materials and methods: To allow exploration of a wide dose range and to avoid hypoglycaemia, doses were given during a euglycaemic clamp, i.e. the subjects were kept at euglycaemic plasma levels (5.6 mM) by continuous glucose infusion. Gradually increasing single oral doses of 2 mg up to 180 mg AZD1656 suspension or placebo were administered during fasting conditions. Each subject participated once, either on AZD1656 or on placebo (3:1 in SAD and 5:1 in JSAD), with the exception of two dose groups in the SAD. Subjects in these two latter dose groups participated on 2 occasions, once on AZD1656/placebo given in fasting conditions and once on AZD1656/placebo given in combination with a standardized breakfast. Seven dose levels were evaluated in the SAD and six in the JSAD. The pharmacokinetic and pharmacodynamic variables were estimated by non compartmental and non-linear mixed effects analyses.

Results: In total, 64 healthy male volunteers from 20 to 45 years of age participated in the studies. AZD1656 was well tolerated in both populations and most adverse events were mild. No treatment related changes in ECG, vital signs or safety lab assessments were observed. Following administration of AZD1656 suspension to fasting subjects, the compound was rapidly absorbed at all dose levels studied. C_{max} was generally reached within 1 hour after intake of dose. Administration of AZD1656 with a high-fat breakfast had no effect on the AZD1656 AUC, while C_{max} was decreased and t_{max} was delayed. In both populations there was a dose-proportional increase in AUC but a somewhat less than dose proportional increase in C_{max} . AZD1656 levels were on average ~25% higher in the Japanese population. The data were best described by a two compartment model with linear elimination and saturable absorption. After scaling of disposition parameters by body weight, the pharmacokinetics were similar between the populations. An active metabolite was formed, with a longer $t_{1/2}$ than AZD1656. However, the exposure to the metabolite was only ~10% of the exposure to AZD1656. A dose-dependent blood glucose lowering effect with AZD1656, of similar magnitude across the two populations, was indirectly demonstrated through a need of increased glucose infusion rate to maintain a constant blood glucose level. Dose-dependent increases in s-insulin and c-peptide were also evident in both populations supporting the anticipated mechanism of action.

Conclusion: AZD1656 was well tolerated in single doses up to 180 mg in both populations and no safety concerns were raised. AZD1656 was rapidly absorbed, and a dose-proportional increase in exposure was observed. Taking differences in body weight into account, there was no difference in the population pharmacokinetic parameters between Western and Japanese subjects. A dose-dependent blood glucose lowering effect, of similar magnitude across the populations, was indirectly demonstrated as well as increased insulin levels.

Clinical Trial Registration Number: NCT00726427; NCT00741689

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Intact central counter-regulatory responses to hypoglycaemia induced by oral glucokinase activators in comparison with insulin infusion in healthy male volunteers

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Background and aims: AZD1656 and AZD 6370 are glucokinase activators (GKA) with an anticipated dual mechanism of action by activating GK in both the liver (decreased glucose output) and pancreas (increased insulin secretion). Dual mechanism GKAs may have the potential to provide efficient glycaemic control in patients with Type 2 diabetes. Because GK is also present in hypothalamic neurons, it has been hypothesized that GK activation could impair the hypothalamic hormone regulated counterregulatory response to hypoglycemia. The aims of the present studies were to assess the counterregulatory responses during a stepwise hypoglycemic glucose clamp in healthy male volunteers following a single oral dose of AZD6370 or AZD1656 in comparison to the counterregulatory response to hypoglycemia during an insulin infusion.

Materials and methods: Both studies used a randomised, open label, two-way cross-over design with at least a two week washout period between glucose clamps. Following an overnight fast, a stepwise hypoglycaemic glucose clamp with target levels of 5 mmol/L (for 60 min), 4 mmol/L (for 30 min), and 3.2 mmol/L (for 60 min) was performed. Plasma glucose was then kept at a nadir of 2.7 mmol/L for 30 min. The glucose clamp was released at 180 min, when blood glucose was allowed to increase to euglycaemia during a 60 minute recovery period. In the AZD 6370 study 12 subjects received either a single oral dose of AZD6370 (300 mg) or an insulin infusion (0.8 mU/kg/min) during the hypoglycaemic clamp. In the AZD1656 study 12 subjects received either a single oral dose of AZD1656 (80 mg) plus supporting insulin infusion or during the second clamp an insulin infusion alone (1 mU/kg/min). The counterregulatory response was assessed based on the glucagon, epinephrine, norepinephrine, growth hormone and cortisol increases.

Results: At the doses applied, only AZD6370 alone (300 mg) was able to lower plasma glucose to the hypoglycaemic target level, despite similar plasma concentrations of unbound/free AZD 6370 and AZD1656. Only by adding a supporting insulin infusion to AZD1656 could plasma glucose be lowered to achieve hypoglycaemia. There was no difference in the counterregulatory response for epinephrine, norepinephrine, growth hormone and cortisol to hypoglycaemia induced by either of the GKAs compared to insulin alone. The glucagon counterregulatory response to hypoglycaemia was blunted with both GKA compounds compared to insulin. Plasma glucagon levels (pmol/L, mean±SD) at baseline and then at a glucose level of 2.7 mmol/L were 33±6 and 32±8 with AZD6370 vs 33±5 and 49±14 with insulin, and 19±10 and 20±9 with AZD1656 vs 17±5 and 31±15 with insulin. The impaired glucagon response is likely due to intra islet hyperinsulinaemia induced by GKAs or by local GK activation of the GK receptors known to be present in the α-cells.

Conclusion: There is no alteration in the CNS hormone and sympathetic nervous system response during hypoglycaemia induced by AZD1656 or AZD6370 compared to insulin alone. However, the glucagon response was blunted, most likely due to direct effects of GKAs on the pancreas.

Clinical Trial Registration Number: NCT00790153

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AZD1656, a novel glucokinase activator, lowers plasma glucose in patients with type 2 diabetes

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Background and aims: AZD1656, a novel glucokinase activator with an anticipated dual mode of action in the liver and pancreas, may have the potential to provide effective blood glucose lowering for patients with diabetes. This multiple ascending dose (MAD) study compared the safety, tolerability, pharmacokinetic (PK) properties and glucose-lowering potential of AZD1656 to placebo in patients with Type 2 Diabetes.

Materials and methods: This randomized, single-blind, placebo-controlled, two part (A & B) phase 1 study was performed in patients with Type 2 Diabetes. Pre-existing oral antidiabetic treatments were washed out one week before randomization. In part A, 32 patients (23 male, 9 female, age 53.7 yrs, BMI 30.8 kg/m²) received either 7, 20, 40 or 80mg bid of AZD1656 or placebo for 8 days. Half the dose was given the first day and, if tolerated, the full dose on the rest of the days. In part B a total of 20 patients (18 male, 2 female, age 50.2 yrs, BMI 32.1 kg/m²) started on 7.6 mg AZD1656 or placebo and over a period of 3 days were up-titrated as tolerated to 15 mg bid or a top dose of 45 mg bid and then treated for another 25 days. A follow-up visit was performed at the end of both parts.

Results: Fifty-one (51) out of 52 patients completed the study; 1 patient in part B withdrew consent. AZD1656 was well tolerated and had a good safety profile across all dose levels. The pharmacokinetics of AZD1656 were, in general, dose- and time-independent although a slightly less than dose proportional increase in C_{max} was observed. Only low amounts of AZD1656 and its equipotent metabolite were excreted in urine. Pharmacodynamic (PD) effects showed plasma glucose lowering with increasing doses of AZD1656 in part A of the study. Fasting plasma glucose (FPG) in the four dosing groups (7, 20, 40, 80 mg) decreased after 8 days by 4%, 6%, 12% and 21% compared to placebo, reaching statistical significance in the 80mg group (95% CI: -36%, -4%). FPG effects were confirmed in part B of the study with a decrease by 21% vs. placebo (95% CI: -39%, 3%). In both part A and B, mean daily (24 h) glucose levels following AZD1656 were rapidly reduced compared to placebo; in part A by up to 24% (-39%, -5%) and in part B by 28% (-46%, -3%). Serum insulin and C-peptide levels did not show any dose related changes in part A nor in part B of the study.

Conclusion: In this placebo-controlled, two part MAD study, AZD1656 was well tolerated when given as monotherapy for up to 28 days in patients with Type 2 Diabetes. At the selected doses, the PK parameters were virtually dose- and time-independent and PD effects were dose-dependent with plasma glucose lowering effects shown at all dose levels above 7 mg bid. Effects on insulin after repeated dosing were inconclusive.

Clinical Trial Registration Number: NCT00747175

Supported by: AstraZeneca

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Single doses of the glucagon receptor antagonist LY2409021 reduce blood glucose in healthy subjects and patients with type 2 diabetes mellitus

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Background: LY2409021 (LY) is a potent, selective antagonist of the glucagon receptor. In T2DM, glucagon levels are inappropriately elevated and contribute to hyperglycemia.

Materials and methods: This Phase 1, randomized, double-blind, placebo (PBO)-controlled, crossover study examined the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of single escalating doses of LY in healthy subjects (HS; 2.5, 10, 30, 100, 250, and 500 mg LY; N=23 males) and patients with T2DM treated with diet and exercise (T2DM; 75, 200, and 500 mg LY; N=9 [5M and 4F]); fasting blood glucose [FBG] 93.6 to 207.0 mg/dL; HbA1c 5.6 to 8.7%). In each dosing period, serial blood samples were collected for measurement of LY PK and PD and standardized meals were provided. Safety was assessed by medical exams, laboratory tests, vital signs, ECGs, and adverse events (AEs).

Results: PK parameters t_{max} , $t_{1/2}$, and apparent clearance (CL/F) were comparable across dose levels and between HS and T2DM, ranging from 4 to 8 h, 50.8 to 58.6 h, and 0.232 to 0.396 L/h, respectively. Plasma exposure of LY increased in proportion to dose in HS and T2DM. Least squares (LS) mean changes from baseline in FBG vs. PBO ranged from +2.9 to -11.5 mg/dL in HS and from -21.9 to -33.3 mg/dL in T2DM. Across LY dose groups in HS, the mean predinner (10-h post-dose) glucose value appeared 0.11 to 11.4 mg/dL lower than PBO; in T2DM, mean premeal glucose values at lunch (4-h), dinner (10-h), and breakfast (24-h), respectively, appeared from 3.6 to 42.9 mg/dL, 8.4 to 29.8 mg/dL, and 14.0 to 48.5 mg/dL lower than PBO. There were no consistent dose-related changes in postprandial glucose. In general, LY reduced fasting serum insulin, with significant LS mean changes vs. PBO ($p \leq 0.02$) observed at higher dose levels (250 and 500 mg in HS at 24-h, and 200 mg [24-h] and 500 mg [48-h] in T2DM). Notably, hypoglycemia did not occur in HS or T2DM after LY administration. There were no significant changes in laboratory tests, vital signs, or ECGs, and review of AEs indicated LY was generally well tolerated.

Conclusion: This first-in-man study demonstrated clinically significant glucose-lowering by single doses of LY in T2DM.

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The glucagon receptor antagonist LY2409021 attenuates increases in hepatic glucose output (HGO) and blood glucose during hyperglucagonaemia in healthy male subjects

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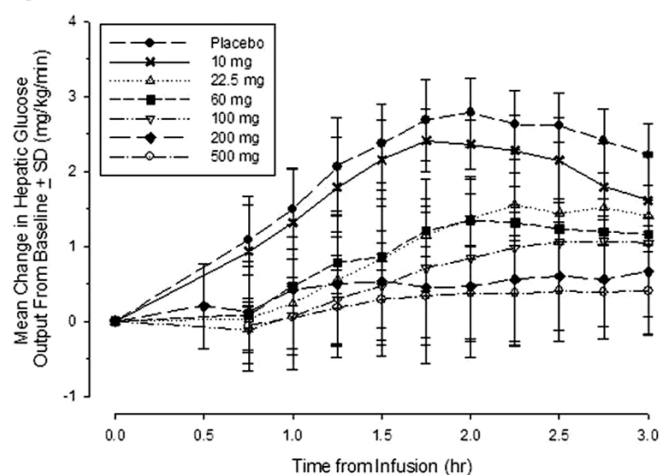
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Background: In type 2 diabetes, glucagon levels are inappropriately elevated, promoting HGO and contributing to hyperglycemia.

Materials and methods: This Phase 1, randomized, subject-blind, placebo (PBO)-controlled, partial crossover study examined the ability of single doses of LY2409021 (LY), a potent, selective glucagon receptor antagonist, to attenuate increases in HGO and blood glucose during hyperglucagonemia in 21 healthy male subjects (HS). Approximately 9 hr after administration of LY or PBO, an infusion of 6,6-[²H₂]glucose was started in fasted HS (5 mg/kg for 3 min, then 0.05 mg/kg/min for the next 6 h), followed 3 hr later by a simultaneous "triple infusion" (TI) of 3 h duration of somatostatin (0.1 µg/kg/min), insulin (0.07 mU/kg/min), and glucagon (6 ng/kg/min) to induce exogenous hyperglucagonemia.

Results: There were no clinically significant variations in laboratory tests or vital signs during the study, and the adverse event profile indicated LY was generally well tolerated. Before the TI, mean HGO rates and blood glucose concentrations were similar across LY dose levels: 2 to 3 mg/kg/min and 70 to 90 mg/dL, respectively. HGO and blood glucose increased approximately 2- to 3-fold during TI; however, LY attenuated increases in both the mean change from baseline in HGO (0-3 hr; normalized to pre-infusion values as shown) and the mean change from baseline in blood glucose concentration (0-3 hr) in a dose-dependent manner: by 15 to 84% and by 11 to 81%, respectively. The effects of LY on HGO and blood glucose were highly correlated ($r=0.97$; $p<0.0001$), indicating that the majority of the glucose-lowering effect of LY was due to an effect on HGO.

Conclusion: These results demonstrate the ability of LY to limit glucagon-induced increases in blood glucose, and support further examination of LY as a potential treatment for T2DM.



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Improved glycaemic control and beta cell function by treatment with TAK-875, a GPR40 agonist, in combination with metformin in Zucker Diabetic fatty rats

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Background and aims: GPR40 is a G protein-coupled receptor highly expressed in pancreatic β -cells that mediates free fatty acid-induced insulin secretion. TAK-875 (TAK) is a selective GPR40 agonist that lowers plasma glucose via stimulation of glucose-dependent insulin secretion. Metformin

(MET) ameliorates insulin resistance by pleiotropic action. Because of the complementary mechanisms of action, the combination of TAK and MET is expected to provide an additive improvement in glycemic control. This study evaluated acute and subchronic effects of TAK plus MET combination in Zucker Diabetic Fatty (ZDF) rats, which spontaneously develop severe diabetes and progressive β -cell dysfunction.

Materials and methods: TAK and MET were dosed by oral gavage. Acute effects of TAK (3mg/kg), MET (50mg/kg), and TAK (3mg/kg) plus MET (50mg/kg) combination treatment on postprandial glucose levels were evaluated by an oral glucose tolerance test (OGTT). In fasted ZDF rats, the acute effects of TAK (10mg/kg), MET (150mg/kg), and TAK (10mg/kg) plus MET (150mg/kg) combination treatment on fasting hyperglycemia were evaluated. Effects of subchronic treatment with TAK (3 and 10 mg/kg, BID), MET (50 mg/kg, QD) and TAK (10 mg/kg, BID) plus MET (50 mg/kg, QD) were evaluated in a 6-week multiple dosing study using ZDF rats.

Results: In an OGTT, oral administration of TAK (3mg/kg) or MET (50mg/kg) decreased glucose AUC by 21.0% or 15.4%, respectively, and TAK (3mg/kg) +MET (50mg/kg) additively decreased glucose AUC by 33.8%. In 19-week old ZDF rats with fasting hyperglycemia (ZDF: 234mg/dL; lean: 101mg/dL), combination treatment with TAK (10mg/kg) and MET (150mg/kg) additively decreased fasting plasma glucose compared with TAK or MET respectively. In a multiple dosing study, combined treatment with TAK (10mg/kg, BID) and MET (50mg/kg, QD) additively decreased glycosylated hemoglobin (TAK: -1.7; MET: -1.8; TAK+MET: -2.4%) and increased fasting plasma insulin (TAK: +7.4; MET: +8.3; TAK+MET: +13.2 ng/mL) compared with TAK or MET respectively. No significant change in fasting plasma glucagon, fasting plasma total cholesterol and fasting plasma NEFA levels were observed with any of the treatments. In addition, in TAK+MET treated ZDF rats, HOMA- β was 9.6-fold higher than control ZDF rats and pancreatic insulin content was maintained to a level comparable to normal littermates (Control: 26; TAK: 42.3; MET: 58.5; TAK+MET: 67.1; normal: 69.1 ng/mg pancreas). These results suggest that combination treatment with TAK and MET has the potential to slow the progression of diabetes and β -cell dysfunction in ZDF rats, which may result from improvements in both postprandial and fasting hyperglycemia.

Conclusion: Our results suggest that TAK plus MET combination therapy may be a valuable strategy for glycemic control and β -cell preservation in patients with type 2 diabetes.

fasting glucose from 500 to 150 mg/dL. Administration of 30 mg/kg AR-7947 significantly decreased serum triglyceride levels 37% after 14 days of dosing. Treatment with AR-7947 at low doses in combination with a DPP4 inhibitor led to 2 fold increased efficacy in an OGTT versus monotherapy.

Conclusion: These studies demonstrate that AR-7947 may show utility as a novel treatment for durable normalization of glucose and lipid profiles in patients with type 2 diabetes.

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AR-7947, a GPR119 agonist with durable reductions in post-prandial and fasted blood glucose in preclinical models of type 2 diabetes

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Background and aims: Agonism of the G-protein coupled receptor, GPR119, results in glucose-dependent incretin release (i.e., GLP-1) from enteroendocrine cells and insulin secretion from pancreatic β -cells. GPR119 agonists have the potential to work additively with DPP4 inhibitors leading to increased active GLP-1 levels, better glucose control and the potential for weight loss in Type II diabetic patients compared to treatment with a DPP4 inhibitor alone. Using traditional medicinal chemistry techniques, AR-7947 was identified as a selective small molecule GPR119 agonist with good physicochemical characteristics and drug-like properties. This presentation describes the *in vitro* and *in vivo* pharmacology of AR-7947.

Materials and methods: A variety of *in vitro* assays including but not limited to cellular EC₅₀, predicted metabolism, permeability, physicochemical determinations, and selectivity panels for kinases, receptors and ion channels were used to characterize AR-7947. This compound was tested *in vivo* in normal mice, rats, dogs, and monkeys to assess PK properties. Finally, C57BL/6 mice and female high fat diet fed ZDF rats were used to show proof of concept and to determine efficacy as monotherapy and in combination with a DPP4 inhibitor. GLP levels, fasting glucose, nonfasted glucose, and AUC_{glucose} in OGTT studies were monitored.

Results: *In vivo* pharmacokinetics of AR-7947 in the rat and monkey showed dose-escalating exposure, high oral bioavailability (up to 59% F) and low clearance. Studies in C57BL/6 mice demonstrated that AR-7947 increased active GLP-1 alone (4 fold) and in combination with a DPP4 inhibitor (12 fold) compared to control. In 14-day studies using female ZDF rats fed a high-fat diet, AR-7947 at 30 mg/kg significantly reduced the glucose excursion in an OGTT 49%. This treatment normalized fasting glucose and lowered non-

PS 076 Innovative concepts in the treatment of type 2 diabetes

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Effects of aleglitazar, a dual PPAR- α/γ agonist, on lipid parameters in patients with type 2 diabetes: post hoc sub-analysis comparing patients with or without statins at baseline

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Background and aims: Many patients with type 2 diabetes (T2DM) also receive treatment for dyslipidemia, such as statins, to help reduce cardiovascular risk factors. However, despite the effects of statin therapy, there remains a substantial residual risk of cardiovascular events. Aleglitazar (ALE; a dual peroxisome proliferator-activated receptor [PPAR]- α/γ agonist) significantly improves glycemic and lipid parameters in patients with T2DM. In SYNCHRONY (N = 332), ALE 150 $\mu\text{g/day}$ (the dose selected for Phase III investigation; n = 55) increased high-density lipoprotein cholesterol (HDL-C) by 21% and decreased triglycerides and low-density lipoprotein cholesterol (LDL-C) by 43% and 15%, respectively from baseline compared with placebo (n = 55), with no adverse cardiovascular effects. ALE 150 $\mu\text{g/day}$ increased apolipoprotein A1 levels and decreased levels of apolipoprotein B compared with placebo. Improvements in all parameters with ALE were numerically greater than with pioglitazone (PIO). Fibrinogen was decreased with ALE and increased with PIO. The post hoc sub-analysis of SYNCHRONY presented here investigated the effects of ALE 150 $\mu\text{g/day}$ on lipids in patients receiving statins at baseline (15% of study population) compared with statin-free patients.

Materials and methods: Least squares mean changes in lipoproteins from baseline to study end in statin-treated versus statin-free patients were assessed using ANCOVA (treatment as fixed effect; baseline value as covariate) in patients randomized to 16 weeks' double-blind treatment with ALE 150 $\mu\text{g/day}$ or placebo, or to open-label PIO 45 mg/day.

Results: ALE treatment reduced LDL-C (-19.80% and -7.29%) and triglycerides (-32.91% and -25.68%) and increased HDL-C (26.15% and 23.30%) in both statin-treated and statin-free groups, respectively (Table), with improvements numerically greater in statin-treated patients. Conversely, PIO increased LDL-C in both statin-treated and statin-free patients.

Conclusion: Despite low patient numbers, these results suggest that ALE may have beneficial effects on lipid parameters in patients with T2DM in addition to those provided by statin therapy.

Effect of placebo, ALE 150 $\mu\text{g/day}$ and PIO 45 mg/day on lipid parameters

	Placebo		ALE 150 $\mu\text{g/day}$		PIO 45 mg/day	
Baseline statin use	Statin-free (n = 45)	Statin-treated (n = 10)	Statin-free (n = 48)	Statin-treated (n = 7)	Statin-free (n = 50)	Statin-treated (n = 7)
Triglycerides						
BL mean, mmol/l	2.57	2.07	2.12	2.79	2.63	2.14
Change from BL, %	17.22	14.56	-25.68	-32.91	-5.34	6.37
LDL-C						
BL mean, mmol/l	3.48	2.86	3.45	2.55	3.59	2.82
Change from BL, %	8.59	2.80	-7.29	-19.80	4.78	25.30
HDL-C						
BL mean, mmol/l	1.18	1.04	1.21	1.31	1.22	1.15
Change from BL, %	3.20	5.37	23.30	26.15	13.06	22.24

BL = baseline.

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LIM-0705: A novel small molecule insulin sensitizer which improves glycemic control in rodent models of insulin resistance/type 2 diabetes and glucose tolerance in healthy volunteers

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Background and aims: Type II diabetes is characterized by peripheral insulin resistance and insulin deficiency. Thiazolidinedione (TZD) drugs, including rosiglitazone, exhibit potent antidiabetic and insulin sensitizing effects but are associated with adverse effects including weight gain and edema. Therefore, insulin sensitizers working through complementary mechanisms are urgently needed. LIM-0705 is a novel insulin sensitizer under development for the treatment of metabolic diseases. In this study, the pharmacological effects of LIM-0705 were examined in vitro, in the Zucker diabetic fatty (ZDF) rat model, in the diet induced obesity (DIO) mouse model and in normal human volunteers.

Materials and methods: The effect of LIM-0705 on glucose production and AMPK activation was evaluated using H4IIE cells (rat hepatoma). Agonist activity against 12 human nuclear hormone receptors including PPAR α , β , δ , γ was evaluated in reporter gene assays. The effects of 42 day i.p. LIM-0705 treatment (114 mg/kg) or rosiglitazone (6 mg/kg) on plasma glucose and lipid metabolism were evaluated in obese ZDF rats (7 wk, 269 g). The effects of 6-8 week i.p. LIM-0705 treatment (100 mg/kg) on glucose regulation and insulin sensitivity was examined in DIO mice (C57BL/6 mice, 18 wk, 47 g) by hyperinsulinemic-euglycemic and hyperglycemic clamp studies. LIM-0705 was studied in a Phase I study with 11 healthy male volunteers (750 mg, BID, p.o.) for 14 days. Metabolic assessments were made for glucose and lipid/cholesterol regulation and by OGTT.

Results: LIM-0705 inhibited glucose production in H4IIE cells (IC_{50} = 6-12 μM) with no cytotoxicity and did not activate AMPK. LIM-0705 showed no agonist activity (<4 fold @ 10 μM) against all 12 nuclear hormone receptors tested. Treatment of ZDF rats with LIM-0705 for 42 days caused rapid and significant reductions in basal glucose levels (501, 207 and 134 mg/dL for vehicle, LIM-0705 and rosiglitazone, respectively, $p < 0.0002$ for both groups compared to vehicle). Day 42 HbA1c levels were significantly reduced (6.1%, 3.3%, and 3.1% for vehicle, LIM-0705 and rosiglitazone, respectively, $p = 0.006$). Rosiglitazone treated rats had a 48% increase in weight compared to vehicle ($p = 0.0007$) while LIM-0705 rats had a 9% ($p = 0.13$) increase. Improvements in basal glucose levels were observed in DIO mice treated with LIM-0705 for 6-8 weeks (-18%, $p = 0.005$), without significant weight gain. Furthermore, under hyperinsulinemic-euglycemic clamp conditions, the glucose infusion (GIR) and insulin stimulated glucose disposal rates (IS-GDR) increased (38.5%, 27.5%, GIR and IS-GDR respectively, $p < 0.03$). In healthy male volunteers who received LIM-0705, 2-hour OGTT glucose values were significantly improved (-12.4% on Day 8, $p = 0.0006$ and -17.1% on Day 14, $p = 0.03$).

Conclusion: LIM-0705 is a novel insulin sensitizer that inhibits glucose production in hepatocytes through a pathway distinct from TZDs and metformin. LIM-0705 treatment produces anti-glycemic effects comparable to rosiglitazone in ZDF rats, improves GIR and IS-GDR under clamp conditions in the DIO mouse, and improves 2-hour OGTT in human volunteers. Chronic dosing of LIM-0705 did not cause significant weight changes compared to vehicle. Further development in insulin resistant and metabolic disease patient populations is warranted.

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The effect of KB003305, a liver selective glucocorticoid receptor antagonist, on fasting plasma glucose and oral glucose tolerance after multiple oral doses in patients with diabetes type 2

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Background and aims: In the fasting state, hepatic glucose output (HGO) is the major glucose source. One of the key hormones that promote HGO is cortisol. KB003305 is a novel, first-in-class anti-diabetic agent for the treatment of type 2 diabetes through liver-targeted antagonism at the glucocorticoid receptor. KB003305 has shown high affinity to the glucocorticoid receptor in

vitro and potent anti-diabetic activity in animal models of diabetes. The aim was to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of repeated oral doses of KB003305 as monotherapy in patients with type 2 diabetes mellitus.

Materials and methods: The design was a randomised, double-blind, placebo controlled, multiple-dose administration, of 100 mg three times daily for 7 days followed by another 7 days of 150 mg 3 times daily. A total of 21 subjects were treated, 14 subjects on active substance and 7 on placebo. Safety variables, pharmacokinetics as well as pharmacodynamic parameters were assessed at baseline (day -2), and after one and two weeks of treatment. Daily fasting plasma glucose (FPG) was measured.

Results: Within-group statistical analysis showed that on treatment with KB003305, FPG was significantly lower than baseline from treatment day 3 and onwards. Placebo treatment FPG levels were higher on all days compared to baseline, but this was statistically significant only for treatment days 5, 13, 14, and 15. Between-group analysis demonstrated significantly lower FPG levels with KB003305 treatment as compared to placebo from treatment day 3 and onwards. Thus, FPG levels were, in comparison to placebo, lowered by 18% on treatment day 7 and by 28% (one-sided 95% CI [$-\infty$, 21%]) on day 14. KB003305 was well tolerated; minor gastrointestinal adverse events occurred mostly during the first treatment days and tended to diminish over time.

Conclusion: KB003305 is a promising new first-in-class anti-diabetic compound with liver-targeted glucocorticoid receptor antagonism which suppresses hepatic glucose production and thus improves fasting plasma glucose without apparent systemic side effects in type 2 diabetic patients.

Clinical Trial Registration Number: EudraCT No.: 2008-004233-16

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Time-action profile of oral enteric insulin in comparison to NPH insulin in Chinese healthy volunteers

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Background and aims: Oral administration of insulin has the potential advantage of a more physiological action by its direct effect on hepatic glucose production. In this study, bio-adhesive calcium phosphate embedded insulin nano-particles with enteric coated capsules were used to facilitate gastrointestinal insulin absorption and overcome gastric acid damage. The aim of the study was to evaluate the pharmacodynamic profiles and duration of action for three oral doses of insulin (50,100 and 200 IU) and one subcutaneous dose of NPH insulin (6 IU).

Materials and methods: This single-center, randomized, four-period, cross-over study was carried out under euglycemic clamp conditions in 12 healthy volunteers. Serum insulin concentrations and glucose infusion rates (GIRs) to keep blood glucose concentrations constantly at 5.0mmol/L were monitored over 12 hours. A low dose infusion of regular insulin (0.15mIU/kg/min) was applied for the entire duration of this euglycemic clamp study to suppress endogenous insulin secretion.

Results: Administration of either NPH or enteric insulin capsules (50,100 and 200IU) increased GIRs. The time for reaching a maximum value of enteric insulin capsules was 250 ± 118 min, 170 ± 58 min, 236 ± 132 min, respectively, versus 243 ± 79 min for NPH. Onset of action (T_{effect}) was longer with capsules of 50IU (38 ± 10 min), 100IU (41 ± 18 min), 200IU (65 ± 58 min) versus NPH (35 ± 8 min). The maximal metabolic activity (GIR_{max}) observed of enteric insulin capsules was lower compared with NPH (GIR_{max} 1.66 ± 0.50 , 1.61 ± 1.00 , 1.80 ± 0.60 , respectively, vs. 2.06 ± 0.82 mg/kg/min). A pronounced peak as same as NPH with enteric insulin capsules was detected in the first 1 hour after administration. The metabolic effect measured over 10 hours tended to be lower with enteric insulin capsules compared with NPH insulin (GIR-AUC₀₋₆₀₀ min 428 ± 269 , 457 ± 254 , 421 ± 332 , vs. 658 ± 405 mg/kg). The relative effectiveness of enteric insulin capsules was 37.0 ± 90.0 , 13.0 ± 27.2 , 11.0 ± 28.8 respectively, vs. 100.0 ± 0.0 of NPH insulin. No dose-response relationship in the absorption and metabolic effect of the enteric insulin capsules was observed among 50IU, 100IU, and 200IU capsules.

Conclusion: Comparison of the pharmacodynamic and pharmacokinetics summary parameters of enteric insulin capsules obtained with Chinese volunteers to NPH insulin, we conclude that: (a) Oral enteric insulin capsules showed a similar time-action profile as NPH insulin. (b) Administration of oral enteric insulin demonstrated an obvious hypoglycemic effect with only a small increase in circulating plasma insulin concentrations. These characteristics discussed above suggest that the possible metabolism of oral enteric

insulin capsule as same as the natural insulin production is limited to the liver with a lower risk of hypoglycemia.

Clinical Trial Registration Number: 2009L09790

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Inhibition of the CCR2 chemokine receptor in diabetic mice results in a rapid and robust improvement of hyperglycaemia

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Background and aims: The role of inflammation in diabetes continues to be elucidated, especially the impact that infiltrating monocytes can have on multiple tissues involved in the disease. Increased adiposity leads to recruitment of inflammatory myeloid cells into adipose tissue and production of factors known to impair systemic insulin sensitivity (i.e., TNF α , IL-6, and MCP-1). Also, myeloid cell recruitment into diabetic liver has been associated with alterations in key metabolic pathways. The current studies were undertaken to address the mechanism by which interventional CCR2 antagonism improves glycemic control in diabetic mice.

Materials and methods: CCX417 (a small molecule CCR2 antagonist, analogue of the clinical compound CCX140-B, which recently completed a Phase 2 clinical trial in type 2 diabetics) was dosed daily to male db/db mice (age 12-19 weeks) for up to 3 weeks. Weekly assessments included body weights, fasting plasma glucose, 24-hr urinary volume and urinary glucose output, and terminal measures included hepatic triglyceride and glycogen content, and hepatic activity of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK).

Results: Treatment with CCX417 for 2 weeks significantly improved glycemic control in both models of type 2 diabetes. CCX417 significantly reduced fasting plasma glucose levels in db/db mice (250 mg/dL vs. 350 mg/dL; $p=0.05$ after 1 week; 300 mg/dL vs. 470 mg/dL; $p=0.03$ after 2 weeks). Treated db/db mice also displayed significant reductions in urinary volume (3.5 mL/day vs. 9.0 mL/day; $p=0.002$) as well as glucosuria after 2 weeks (1000 mg/day vs. 1500 mg/day; $p=0.05$). Systemic lowering of glucose levels coincided with a reduction in hepatic G6Pase activity after 2 weeks (12 μ mol/min/g vs. 18 μ mol/min/g; $p=0.001$). Improved glycemic control also coincided with reductions in hepatic triglyceride levels (2.2 mg/g vs. 4.3 mg/g; $p=0.02$).

Conclusion: Robust and rapid improvements of hyperglycemia and glucosuria were seen following pharmacological intervention with a small-molecule CCR2 antagonist in a mouse model of type 2 diabetes. These results support the continued clinical evaluation of CCR2 antagonists, such as CCX140-B, for the treatment of type 2 diabetes and diabetic co-morbidities such as hepatic steatosis.

CCR2 Antagonist Effect in Diabetic db/db Mice After 2 Weeks of Treatment

	Vehicle Treated Mice	CCR2 Antagonist Treated Mice	p-value
Fasting Blood Glucose (mg/dL)	470	300	0.03
Urinary Volume (mL/day)	9.0	3.5	0.002
Urinary Glucose (mg/day)	1,500	1,000	0.05
Hepatic G6Pase Activity (μ mol/min/g)	18	12	0.001
Hepatic Triglycerides (mg/g)	4.3	2.2	0.02

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Safety and efficacy of monthly s.c. canakinumab administration for the treatment of hyperglycaemia in metformin monotherapy-treated type 2 diabetic patients

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Background and aims: Canakinumab is a human monoclonal anti-human IL-1 β antibody of the IgG1/k isotype that binds to human IL-1 β and thus blocks the interaction of this cytokine with its receptors. This action potentially reduces inflammation thereby preserving/improving β -cell function. The purpose of this study was to determine the optimal dose of canakinumab when dosed once a month s.c. to improve blood glucose control in metformin-treated patients with type 2 diabetes mellitus.

Materials and methods: The primary objectives were to assess the effect on HbA_{1c}, and the safety and tolerability of four doses of canakinumab (5 mg, 15 mg, 50 mg, and 150 mg) vs. placebo as an add-on regimen to metformin over 4 months. Total enrollment in the study included 551 patients (canakinumab=372, placebo=179) with a mean age: 54.1 years (range=28–74 years), 56.4% male, a mean weight (SD) of 81.1 (20.05) kg, a mean BMI of 29.8 kg/m², and a mean HbA_{1c} at baseline of 7.4%.

Results: Modest improvement in HbA_{1c}, at each dose of canakinumab with maximum benefit at the 50-mg monthly dose, is shown in the table. Table: Between-treatment analysis (ANCOVA) of the least squares mean change from baseline in the HbA_{1c} (%) at month 4

	Canakinumab 5 mg	Canakinumab 15 mg	Canakinumab 50 mg	Canakinumab 150 mg	Placebo
Change from baseline	-0.20	-0.29	-0.31	-0.25	-0.13
Placebo- adjusted change from baseline	-0.06	-0.16	-0.18	-0.12	

Safety results showed that canakinumab treatment was safe and well tolerated. The most common adverse events were nasopharyngitis, urinary tract infections, and upper respiratory tract infections. There were no significant differences in adverse events between the canakinumab and placebo groups.

Conclusion: Canakinumab lowered HbA_{1c} modestly, approximately -0.20% (placebo adjusted) from a baseline of ≈7.4%. This confirms that blocking IL-1 β -mediated inflammation by canakinumab results in small improvements in glycemic control, potentially improving β -cell function. There was no dose response detected between active canakinumab doses, but all doses lowered HbA_{1c} between -0.20% to -0.31% (-0.06% to -0.18% in placebo-adjusted analysis). No new safety signals were seen in this study. The safety and tolerability profile is consistent with the known safety experience of canakinumab in other trials.

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IL-1 β antibody (canakinumab) improves insulin secretion rates in subjects with impaired glucose tolerance and type 2 diabetes treated with differing diabetes therapies

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Background and aims: Canakinumab is a human monoclonal anti-human IL-1 β antibody of the IgG1/k isotype that binds to human IL-1 β and thus blocks the interaction of this cytokine with its receptors, thereby neutralizing the activity of IL-1 β . IL-1 β -mediated inflammation has been implicated in suppression of insulin secretion and worsening β -cell function in patients with diabetes. The primary objective of this trial was to evaluate canakinumab effects on change from baseline of meal challenge-derived insulin secretion rate (ISR) relative to glucose.

Materials and methods: Subjects were enrolled in the following type 2 diabetes mellitus (T2DM) populations: 1) on stable metformin monotherapy, 2) on a stable metformin dose in combination with a sulfonylurea (SU), 3) on metformin in combination with an SU and a thiazolidinedione (TZD), 4) on at least two insulin injections a day with or without metformin, and subjects with impaired glucose tolerance (IGT). Subjects received a single dose of 150 mg canakinumab s.c. at week 0 and underwent a standard meal challenge test at weeks 0 and 4. Subjects were randomized either to canakinumab 150 mg s.c. or placebo in a 2:1 ratio except for IGT subjects who were randomized 1:1.

Results: Randomized set: 246 subjects were analyzed (canakinumab=154, placebo=92). There were 126 subjects with T2DM and 28 subjects with IGT treated with canakinumab, and 65 subjects with T2DM and 27 subjects with IGT who received placebo. The mean age of all subjects was 57.4 years (54% male), the mean baseline HbA_{1c} for subjects with T2DM was 7.1% and the mean HbA_{1c} for subjects with IGT was 6.1%. The mean duration of T2DM was 9.4 years. The results of the change in ISR relative to glucose at week 4 from baseline are shown in the table.

Treatment arm	Canakinumab minus placebo (0 to 30 min) pmol/min/m ² /mmol/L	Canakinumab minus placebo (0 to 60 min) pmol/min/m ² /mmol/L	Canakinumab minus placebo (0 to 2 h) pmol/min/m ² /mmol/L*	P Value
Metformin alone	-1.40	0.42	0.17	
Metformin + SU	-0.18	-0.11	-0.41	
Metformin + SU +TZD	-0.43	-1.29	-1.95	
Insulin 2/day \pm Metformin	3.81†	2.70	1.72	†p=0.0525
IGT	3.92‡	2.54	0.43	‡p=0.1729

*Primary endpoint.

Modest increase in ISR relative to glucose (0–30 min) reflecting first-phase insulin secretion was observed in insulin-treated T2DM and in IGT groups. Other groups did not show consistent effects on ISR relative to glucose. There were no deaths or serious adverse events (AEs) in this single-dose study. The most frequently reported AEs (at least 2 subjects in any treatment group) were tremor (6 subjects, 2.5%), hyperhidrosis (5 subjects, 2.0%), dizziness (4 subjects, 1.6%), hypoglycemia (4 subjects, 1.6%) and nasopharyngitis (3 subjects, 1.2%). The safety assessment of canakinumab in this study is consistent with the favorable safety and tolerability seen in other trials.

Conclusion: In conclusion, insulin-treated subjects, whether using metformin or not, as well as IGT subjects, had a trend toward improving ISR relative to glucose (0–30 min) when treated with canakinumab. This result supports the hypothesis that blocking IL-1 β in pancreatic islets has the potential to improve β -cell function by reducing suppression of insulin secretion by IL-1 β -mediated inflammation in these subgroups.

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Pharmacokinetic (PK) and pharmacodynamic (PD) modelling of subcutaneous (SC) LY2189102, a neutralising IL-1 β antibody, in patients with type 2 diabetes mellitus

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Background and aims: LY2189102, a humanized neutralizing IL-1 β antibody, was studied in type 2 diabetes mellitus (T2DM) patients with C-reactive protein (CRP) \geq 2 mg/L, who received weekly subcutaneous doses of LY2189102 (0.6, 18 or 180 mg) or placebo over 12 weeks, and were monitored for 12 additional weeks. This report describes the selection of dose level and frequency of LY2189102 treatment using modeling and simulation of LY2189102 PK/PD relationships.

Materials and methods: Data from 106 patients were used in this analysis. A 2-compartment mammillary model with dose-dependent first-order input and bioavailability was fitted to pooled PK data from this study and a previous study conducted in rheumatoid arthritis patients receiving LY2189102 intravenously. Bioavailability (F) was coded as $F = 1 / (1 + \exp(-(F_A + F_S / \text{Dose})))$, and the absorption half-life (T_{ka}) was coded as $T_{ka} = \alpha \cdot F$, where F_A , F_S , and α were estimated parameters, and Dose is in mg. Concentrations predicted using the individual Bayesian estimates of PK parameters were used as a forcing function in the PK/PD model, which simultaneously fitted fasting glycemia, insulinemia and HbA_{1c} data. In this model, glucose-dependent insulin secretion capacity (ISC) had a zero-order input and LY2189102-concentration-dependent (inhibitory E_{max}) first-order output. Glycemia and insulinemia were interdependently related through ISC and fixed insulin sensitivity and liver glucose output parameters. HbA_{1c} was governed by an input rate that is dependent on glycemia and unglycated hemoglobin, and a first order output. Simulations of a wide range of doses and administration frequencies, from once weekly to once every 6 weeks, were conducted.

Results: Data from 5 patients whose PK profiles indicated presumed development of immunogenicity were excluded. The total clearance (CL), distributional clearance (Q), central volume (V_c), peripheral volume (V_p), F_A , F_S and α were (estimate (%SEM)) 9.45 (4.4%) mL/h, 25.1 (16%) mL/h, 3.06 (7.7%) L, 1.91 (7.7%) L, -0.42 (25%), 0.837 (21.1%) and 217 (10.9%) h, respectively. Inter-individual variability in CL, V_c, V_p and T_{ka} was 44.2%, 51.9%, 69.2%, and 57.9%, respectively. Placebo effect, LY2189102's maximum inhibition of insu-

lin secretion output (I_{\max}), the concentration at 50% effect (IC₅₀), and insulin secretion loss rate constant were 6.5% (45%), 10.4% (39%), 342 $\mu\text{g/L}$ (277%), and 4.72 mo^{-1} (37%), respectively. Inter-subject variability in PD parameters was modest. Simulation, conditional on final estimates, showed that while total administered doses saturably determined the magnitude of response, all studied administration schedules were essentially equivalent, probably due to the long half-life of the compound. A dose of 60 mg, administered once every 6 weeks, sustains near maximum glycemic response (-0.9 mM from baseline, -0.55 mM from placebo), while an 18 mg dose sustains 75% of the maximal response. Glycemic response plateaus by 3 months, while the HbA_{1c} response at 6 months was 14% higher than that at 3 months.

Conclusion: Dosing (SC) of LY2189102 can be as infrequent as once every 6 weeks, potentially offering a convenient therapeutic alternative for patients with T2DM.

Clinical Trial Registration Number: NCT00942188

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Safety, tolerability and efficacy of subcutaneous (SC) LY2189102, a neutralising IL-1 β antibody, in patients (Pts) with type 2 diabetes

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Background and aims: Inflammation is associated with pancreatic beta cell apoptosis and reduced insulin sensitivity. Literature suggests IL-1 β could be a primary contributor to type 2 diabetes (T2DM). This study aimed to determine the efficacy, safety, and tolerability of LY2189102, a humanized neutralizing IL-1 β antibody, in T2DM Pts.

Materials and methods: This was a randomized, double-blind, placebo (pbo) controlled, parallel design study which enrolled 106 Pts with T2DM on diet and exercise, with or without anti-diabetic medication (excluding thiazolidinediones [TZD] and insulin). Pts equally randomized to LY (0.6, 18, 180 mg) or pbo received weekly SC doses for 12 weeks (wks; 13 total doses) with an additional 12 wks follow-up. The primary objective was the change from baseline (CFB) in HbA_{1c} after 12 wks of dosing for the compliant set (≥ 11 doses). Sample size based on 1-sided 75% confidence interval (CI) in CFB between LY and pbo being $\leq -0.68\%$ HbA_{1c}.

Results: All 3 LY2189102 dose groups showed significant (based on 75% 1-sided CI) reduction in HbA_{1c} at 12 wks compared to pbo (Table). Numerical reduction in HbA_{1c} remained evident at the end of 24 wks compared to pbo. There was no obvious dose-response relationship. Fasting glucose CFB was significantly reduced compared to pbo at multiple timepoints and numerical estimates increased closer to baseline at 24 wks. No significant differences were seen in response among predefined ligand and receptor genotypic variants. A significant ($p < 0.05$) and early anti-inflammatory effect was demonstrated, best reflected by C-reactive protein (CRP) response, and appeared saturated and sustained at end of follow-up. Both IL-6 and PAI-1 showed a dose-related numerical reduction. LY2189102 was well tolerated with 4 treatment emergent (TE) serious adverse events (SAEs) in 2 Pts (0.6mg, 18mg), none considered to be drug-related. Five Pts (3 at 18mg, 2 at 180mg) were discontinued due to TEAEs. A similar percentage of Pts reported at least 1 TEAE across all treatment groups. The incidence of TEAEs associated with Infections and Infestations across treatment groups was 34.6%, 23.1%, 22.2% and 18.5% for 0.6, 18, 180 mg and pbo, respectively.

Conclusion: LY2189102 given SC weekly for 12 wks was well tolerated, modestly reduced HbA_{1c} and blood glucose, and demonstrated significant anti-inflammatory effects in T2DM Pts. Neutralizing IL-1 β holds promise as an adjuvant treatment for T2DM.

Table 1

	Placebo	0.6mg LY2189102	18mg LY2189102	180mg LY2189102
	n=23	n=21	n=16	n=19
Baseline				
Mean (SD)	7.824 (0.6557)	7.540 (0.5596)	7.950 (0.7002)	8.271 (0.9380)
End-of-Dosing				
Mean (SD)	7.635 (0.4994)	7.169 (0.5806)	7.378 (0.7134)	7.774 (0.9724)
End-of-Dosing CFB				
LS Mean (SE)	-0.183 (0.1315)	-0.457 (0.1454)	-0.561 (0.1530)	-0.428 (0.1379)
LS Mean Difference	-	-0.274	-0.378	-0.245
75% 1-sided CI	-	(-0.09)	(-0.16)	(-0.04)
p value	-	0.095	0.045	0.168

Clinical Trial Registration Number: NCT00942188

PS 077 From IGT to type 2 diabetes: pathways and prevention

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Risk factors of conversion from IGT and IGF to diabetes type 2 middle-aged population

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Background and aims: To determine the predictive risk factors for the development of type 2 diabetes mellitus (DM) in patients with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) during the 8,5 follow up.

Materials and methods: This is an eight year prospective study in a randomly selected urban population including 2838 subjects aged ≥ 40 years living in Krakow. 706 had IGT (387) or IFG (319) based on WHO criteria and 264 of them attended the follow-up assessment eight years later. Subjects underwent a physical examination including weight/height, waist circumference, biochemical examination including glucose, insulin in 0', 120' OGTT and questionnaire examination concerning CVD health history and family history of type 2 diabetes.

Results: The prevalence of type 2 diabetes in examined population of 264 people with previously (1998-2000) found IGT or IFG was 17%. Diabetes type 2 was found in 22% of people with previously diagnosed IFG and in 13 % of people with previously found IGT. Among people with diagnosed diabetes, 51% had newly diagnosed diabetes during the control study, in 49 % participants diabetes was diagnosed before in the period between the baseline and control study. In the studied population statistically significant predictive factors of the progression to type 2 diabetes were fasting glycemia (RR 3,27; $p < 0,001$), fasting hiperinsulinemia (RR 2,1; $p < 0,05$), insulin resistance measured as HOMA IR index (RR 1,99; $p < 0,05$), WHR, (RR 1,67, $p < 0,01$) and family history of type 2 diabetes (RR 2,21; $p = 0,01$).

Conclusion: In the studied population important predictive factors of the progression to type 2 diabetes were fasting glycemia, fasting hiperinsulinemia, insulin resistance measured as HOMA IR index, WHR, and family history of type 2 diabetes.

Risk of diabetes			
baseline	RR	95% CI	p
BMI	1,04	0,97-1,10	ns
fasting glucose (mmol/l)	3,27	1,62-6,59	0,0009
glucose in 120min DTTG (mmol/l)	0,94	0,79-1,12	ns
fasting insulin (uj/ml)	1,02	0,98-1,06	ns
hiperinsulinaemia	2,1	1,08-4,02	0,028
inlusin in 120 min DTTG (uj/ml)	1,00	1,0-1,01	ns
hiperinsuliaemia in 120min DTTG	1,2	0,65-2,26	ns
HOMA	1,11	0,97-1,28	ns

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Potential impact of elevated HbA_{1c} and impaired fasting glucose on population for predicting risk of diabetes: the Toranomon Hospital Health Management Center Study

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Background and aims: Although evidence has suggested that individuals with both impaired fasting glucose (IFG) and elevated HbA_{1c} have a markedly increased risk of diabetes, the potential impact of these factors among the total population, including the effects of the markedly increased risk and the prevalence of individuals classified as having pre-diabetes, has not been clarified. We therefore tested the hypothesis that, although the number of pre-diabetic individuals identified by both IFG and elevated HbA_{1c} criteria may be small, the markedly increased risk effectively contributes to the prediction of future diabetes, in a large cohort of Japanese individuals.

Material and methods: The study enrolled 4670 men and 1571 women aged 24-82 years without diabetes (diabetes: fasting plasma glucose (FPG) ≥ 126 mg/dl, HbA_{1c} $\geq 6.5\%$, or self-reported clinician-diagnosis diabetes). Baseline diagnosis of pre-diabetes was made according to IFG (FPG 100-125 mg/dl, IFG100; or FPG 110-125 mg/dl, IFG110) or elevated HbA_{1c} (5.7-6.4%, HbA_{1c}5.7; or 6.0-6.4%, HbA_{1c}6.0). We used a combination of two tests with alternative cut-offs for diagnosing pre-diabetes. The potential impact on the study population, including the effects of risk and prevalence, was evaluated by population attribute risk percent (PAR%). The follow-up duration was a median of 5.0 years on an annual basis, during which 338 incident cases of diabetes occurred.

Results: PAR% was 68% for IFG100, 44% for IFG110, 50% for HbA_{1c}5.7%, and 31% for HbA_{1c}6.0%, when each criterion was used for screening as a single test. Among the 6241 study individuals, 410 fulfilled (1) both HbA_{1c}5.7 and IFG100; 135 individuals fulfilled (2) both HbA_{1c}6.0 and IFG100; 159 individuals fulfilled (3) both HbA_{1c}5.7 and IFG110; and only 72 individuals fulfilled (4) both HbA_{1c}6.0 and IFG110. Multivariate adjusted Cox analysis including age, sex, smoking habit, parental history of diabetes, BMI, systolic blood pressure, HDL cholesterol and triglycerides demonstrated that, as compared with normoglycemic individuals, those who fulfilled (1), (2), (3) or (4) had a hazard ratio (HR) of 32.5 (95% CI, 23.0-45.8), 53.7 (38.4-75.1), 37.9 (28.1-51.1), and 52.3 (37.8-72.3) times more increased risk of diabetes, respectively. We observed that the markedly increased risk contributed to a large PAR%; that is, individuals who fulfilled (1), (2), (3) and (4) showed a PAR% of 67%, 53%, 48%, and 37%, respectively (Table 1).

Conclusion: Although the number of pre-diabetic individuals identified by overlap of IFG and elevated HbA_{1c} was small, targeted screening of those with a markedly increased risk could contribute to improve screening efficacy among the total subject population.

Table 1: Hazard ratios for the development of type 2 diabetes according to the baseline diagnosis of pre-diabetes using various cut-offs

Baseline diagnosis of pre-diabetes	n	Age and sex adjusted HR (95% CI)	Multivariate adjusted HR (95% CI)	PAR (%)
IFG100/HbA _{1c} 5.7				
Nomoglycemia	4149	1.00 (Ref.)	1.00 (Ref.)	-
IFG100 alone	1270	6.86 (4.84-9.71)	6.18 (4.34-8.80)	51
HbA _{1c} 5.7 alone	412	6.53 (4.10-10.4)	6.05 (3.79-9.64)	25
IFG100 and HbA _{1c} 5.7	410	38.6 (27.6-54.0)	32.5 (23.0-45.8)	67

IFG100/HbA _{1c} 6.0				
Nomoglycemia	4493	1.00 (Ref.)	1.00 (Ref.)	-
IFG100 alone	1545	6.79 (5.11-9.04)	5.97 (4.46-7.99)	55
HbA _{1c} 6.0 alone	68	8.25 (4.10-16.6)	7.42 (3.67-15.0)	7
IFG100 and HbA _{1c} 6.0	135	65.5 (47.2-90.8)	53.7 (38.4-75.1)	53

IFG110/HbA _{1c} 5.7				
Nomoglycemia	5198	1.00 (Ref.)	1.00 (Ref.)	-
IFG110 alone	221	12.5 (8.93-17.4)	11.4 (8.09-16.1)	27
HbA _{1c} 5.7 alone	663	6.75 (5.01-9.09)	6.26 (4.63-8.45)	36
IFG110 and HbA _{1c} 5.7	159	45.8 (34.5-60.8)	37.9 (28.1-51.1)	48

IFG110/HbA _{1c} 6.0				
Nomoglycemia	5730	1.00 (Ref.)	1.00 (Ref.)	-
IFG110 alone	308	12.3 (9.48-16.1)	10.8 (8.20-14.2)	33
HbA _{1c} 6.0 alone	131	12.5 (8.73-18.0)	11.5 (7.93-16.5)	18
IFG110 and HbA _{1c} 6.0	72	63.3 (46.4-86.5)	52.3 (37.8-72.3)	37

Population attribute risk percent (PAR%): $(P_e [Multivariate adjusted HR-1]) / (P_e [Multivariate adjusted HR-1] + 1)$, where P_e = prevalence of pre-diabetes screened by each criteria.

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Serum levels of vitamin D and abnormal glucose regulation

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Background and aims: Vitamin D deficiency has been suggested to increase the risk of type 2 diabetes (T2D), but data from prospective studies are scarce and equivocal. The aim of this study was to investigate if development of prediabetes (IFG, IGT) and T2D can be predicted by vitamin D, 25(OH)D, levels. Moreover, we evaluated associations between serum 25(OH)D levels and prediabetes.

Material and methods: The study included 980 women and 1398 men, aged 35–56 years at baseline (1992–98), residing in Stockholm County and participating in a prospective population-based investigation. The subjects were examined by oral glucose tolerance test (OGTT) at baseline and follow up 8–10 years later, classifying the subjects as having normal glucose tolerance (NGT), prediabetes or T2D. Moreover, anthropometric measures, blood pressure and data on lifestyle issues were collected and serum concentrations of 25(OH)D were measured at baseline. At baseline, none had previously diagnosed T2D. All subjects having prediabetes or T2D at follow up were selected as cases. The controls were matched by age and sex to cases by random in a group of subjects having NGT at both baseline and follow up. The association between 25(OH)D levels (adjusted for seasonal variation) and prediabetes/T2D was evaluated by logistic regression analyses, controlling for confounders (age, BMI, FHD, blood pressure, physical activity).

Results: Subjects in the highest quartile of 25(OH)D levels were at baseline, more physically active, had lower 2-h glucose in OGTT, lower BMI and waist circumference compared to subjects in lower quartiles. In the prospective study, men but not women in the highest quartile of serum 25(OH)D had a decreased odds ratio of developing T2D after adjustments (OR=0.57; 95% CI 0.33–0.97). This effect was mainly accounted for by subjects with prediabetes at baseline. Development of prediabetes was not associated with 25(OH)D levels. At baseline, subjects in the highest quartile of serum 25(OH)D had a decreased risk of having prediabetes, however significant only in men after adjustments (OR=0.41, 95% CI 0.25–0.68).

Conclusion: High levels of serum 25(OH)D predict reduced risk of developing T2D in men but not in women. Furthermore, there is a cross-sectional association between prediabetes and low serum 25(OH)D levels mainly in men. Our data suggest that vitamin D supplementation could play a role in prevention of T2D in subjects with prediabetes.

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The FINDRISK score is associated with an increase of artery stiffness in prediabetic subjectsA.H. Belhadj Mostefa¹, F. Touati¹, D. Roula¹, P. Valensi²;¹Departement of internal médecine, Constantine, Algeria, ²Departement of Endocrinology, Diabetology, Nutrition, Bondy, France.

Background and aim: Prediabetes is associated with an increased cardiovascular risk. Artery stiffness is an early marker of cardiovascular risk and has been shown to be increased in patients with type 2 diabetes (T2D). The aim was here to look for an association between artery stiffness and the Findrisk score in patients at risk of diabetes.

Materials and methods: We included 222 (166 women) subjects without known dysglycemia but at risk of diabetes (IDF criteria), mean age 50.4 ± 11.4 yrs, BMI 31.9 ± 5.4 kg/m², 65.5% hypertensives. An OGTT was performed, the Findrisk score was calculated, and carotid-femoral pulse wave velocity (PWV) was measured (Complior®).

Results: The prevalence of normal glucose tolerance (NGT), prediabetes (impaired fasting glucose and/or impaired glucose tolerance) and T2D (WHO criteria) was 52%, 29.1% and 18.9%, respectively. A Findrisk score ≥ 14 was strongly associated with current T2D ($p < 10^{-5}$). PWV was higher in T2D and prediabetic patients (10.9 ± 2.9 and 10.6 ± 2.4 m/s) than in NGT patients (9.8 ± 2.5 m/s) ($p < 0.01$). PWV correlated with age and Findrisk score ($p < 10^{-5}$ for both) and was higher in hypertensives than in normotensives ($p < 10^{-5}$). In multivariate analysis, PWV was associated with age and hypertension ($p < 10^{-5}$ in more subjects with a Findrisk score ≥ 14 than in those with a score < 14 (31% vs 6.4%, $p = 0.01$).

Conclusion: In this population at high risk of diabetes, a Findrisk score of 14 or higher is associated with an increase in artery stiffness, even in normotensive subjects. The Findrisk score predicts both current unknown T2D and an increased artery stiffness.

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Lifestyle modification regulates the expression of glucotransporter 4 (GLUT-4) in prediabetic subjects (PS) and subjects with positive family history of type 2 diabetes (FHS)M.A. Bernat-Karpińska¹, A. Czech¹, P.J. Piątkiewicz¹, P. Wierzbicki², M. Nowaczyk²;¹Department of Internal Diseases and Diabetology, ²Department of Clinical Immunology, Medical University of Warsaw, Poland.

Background and aims: The impairment of cellular glucose transport in insulin resistance state leads to glucose metabolism disturbances. The proper expression of particular GLUT isoforms determines a sufficient glucose supply for tissues. The aim of the study was to evaluate the influence of lifestyle modification on insulin resistance markers and the quantitative expression of GLUT-1, GLUT-3 (typical isoforms for peripheral blood cells) and GLUT-4 (an insulin-dependent isoform) on peripheral blood lymphocytes (PBL) in prediabetic subjects and individuals with positive family history of type 2 diabetes (first-degree relatives) during 24 months of observation.

Materials and methods: The study included 25 PS (diagnosed according to WHO criteria) and 24 normoglycemic FHS. 23 healthy individuals with no family history of type 2 diabetes, matched with age, sex and BMI served as a control group. All participants were treated with diet and exercise intervention at least 140 min. per week. The PBL demonstrating expression of GLUT-1, GLUT-3 and GLUT-4 were labeled with the use of indirect immunofluorescence. The expression of GLUT isoforms was investigated by flow cytometry. Cells were stained by using anti-human GLUT antibodies and FITC-conjugated immunoglobulin. Flow cytometry was performed utilizing a FACSCalibur (Becton-Dickinson). The data was analyzed using Cell Quest software and presented as a percentage of lymphocytes revealing expression of the determined receptor proteins. Additionally we determined: BMI, WHR, fasting plasma glucose (FPG), insulin and C peptide (radioimmunological method), HOMA-IR. All the tests were performed at baseline, after 12 and 24 months.

Results: At baseline PS and FHS were characterized of much higher expression of GLUT-4 compared to control subjects. 24 months of lifestyle modification resulted in significant lowering of the expression of GLUT-4 on the surface of PBL in both studied groups, with no differences in the expression of GLUT-1 and GLUT-3. Both PS and FHS revealed no significant differences in determined insulin resistance markers after 24 months of the observation in comparison to the baseline values. The results were compared using the Aspin-Welch test (Table).

Conclusion: The estimation of typical GLUT isoforms present on the PBL as well as the evaluation of insulin resistance markers are insufficient for monitoring the metabolic disorders progression in PS and FHS. The decrease in GLUT-4 lymphocyte expression proves a positive influence of lifestyle modification on a tissue redistribution of this crucial insulin-dependent glucotransporter. The determination of GLUT-4 on the surface of the PBL can be a useful tool for the evaluation of the therapeutic actions efficacy in subjects at high risk of type 2 diabetes.

Table

Parameter (x±SD)	Control group	FHS at baseline	FHS after 12 months	FHS after 24 months	p-values FHS (baseline vs 24 months)	PS at baseline	PS after 12 months	PS after 24 months	p-values PS (baseline vs 24 months)
BMI (kg/m ²)	30,66 ± 3,0	29,50 ± 4,55	29,92 ± 4,58	29,87 ± 4,45	ns	30,14 ± 5,11	30,59 ± 5,8	30,80 ± 5,61	ns
WHR	0,88 ± 0,09	0,87 ± 0,11	0,88 ± 0,1	0,88 ± 0,11	ns	0,87 ± 0,06	0,89 ± 0,07	0,90 ± 0,07	ns
FPG (mmol/l)	5,27 ± 0,4	5,19 ± 0,3	5,63 ± 0,45	5,38 ± 0,58	ns	6,14 ± 0,53	6,31 ± 1,28	6,49 ± 0,88	ns
Insulin (mU/l)	3,49 ± 2,51	10,13 ± 4,74	9,48 ± 6,42	9,61 ± 4,08	ns	13,05 ± 7,51	11,29 ± 11,8	13,02 ± 8,86	ns
HOMA	0,82 ± 0,62	2,34 ± 1,14	2,4 ± 1,66	2,34 ± 1,14	ns	3,52 ± 1,94	3,33 ± 3,63	3,93 ± 3,37	ns
C-peptide (ng/ml)	1,96 ± 0,56	2,35 ± 0,84	2,4 ± 1,66	2,57 ± 0,63	ns	2,81 ± 1,09	2,6 ± 1,11	3,28 ± 1,7	ns
GLUT 1 (%)	23,62 ± 13,87	29,7 ± 10,36	32,47 ± 15,27	30,62 ± 11,72	ns	33,42 ± 13,38	30,53 ± 12,44	31,53 ± 14,1	ns
GLUT 3 (%)	7,44 ± 4,33	7,54 ± 3,3	8,82 ± 6,24	8,24 ± 8,24	ns	8,22 ± 3,84	8,65 ± 6,85	7,48 ± 3,75	ns
GLUT 4 (%)	3,43 ± 2,77	18,93 ± 12,71	13,0 ± 7,44	9,35 ± 6,07	0,01	20,21 ± 14,44	11,54 ± 9,99	9,65 ± 5,73	0,01

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The 10-year cost-effectiveness of lifestyle intervention or metformin for the primary prevention of type 2 diabetes mellitus: an intent-to-treat analysis of diabetes prevention

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The Diabetes Prevention Program (DPP) randomized overweight adults with impaired glucose tolerance (IGT) and an elevated fasting glucose to intensive lifestyle (ILS), metformin (MET), or placebo (PBO) for an average of 3 years. The DPP Outcomes Study (DPPOS) followed participants for an additional 7 years during which time ILS and MET participants were encouraged to continue those interventions and all participants were offered a modified lifestyle intervention. A recent analysis demonstrated that the beneficial effects of ILS and MET on the incidence of type 2 diabetes persisted for at least 10 years after randomization. During both DPP and DPPOS, data on resource utilization, cost, and quality-of-life were collected prospectively. Economic analyses were performed from a health system perspective that considered direct medical costs. During DPPOS, the direct medical costs of ILS and MET were substantially lower than during DPP, and the costs of PBO were higher than during DPP. Over 10 years, the cumulative, undiscounted, per capita direct medical costs of the interventions were greater for ILS and MET than for PBO (\$4,826 ILS vs. \$2,489 MET vs. \$953 PBO). The direct medical costs of care outside the DPP/DPPOS increased over time for all groups, but were highest for PBO. The cumulative undiscounted, per capita, direct medical costs of non-intervention-related medical care were greater for PBO (\$31,299) than MET (\$26,351) or ILS (\$24,759). Over 10 years, the undiscounted per capita total direct medical costs were lower for both ILS (\$29,585) and MET (\$27,840) compared to PBO (\$32,252). Quality-of-life was better for ILS compared to MET or PBO and the undiscounted quality-adjusted life-years accrued over 10 years were greater for ILS (6.81) than MET (6.69) or PBO (6.67 QALYs). Over 10 years, from a payer perspective, ILS and MET were less expensive and more effective than PBO. Both health policy and social policy should support the funding of intensive lifestyle and metformin for diabetes prevention in high-risk adults.

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Long-term weight loss with controlled-release phentermine/topiramate reverses metabolic syndrome and improves associated traits

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Background and aims: Abdominal obesity is one of the key features of metabolic syndrome (MetS), and it correlates directly with the associated risk factors. Reducing weight has been shown to decrease the risk of developing MetS and its component comorbidities. In the 56-week CONQUER study, low-dose, controlled-release phentermine/topiramate (PHEN/TPM CR), when added to lifestyle interventions, demonstrated significant weight loss and resolution of MetS. SEQUEL, a 52-week extension study in subjects completing CONQUER, evaluated the long-term effects of PHEN/TPM CR on weight loss and the components of MetS.

Materials and methods: SEQUEL (N=675) maintained the original randomization and blinding of CONQUER (placebo, PHEN 7.5 mg/TPM CR 46 mg [7.5/46], and PHEN 15 mg/TPM CR 92 mg [15/92]). This subanalysis evaluated weight loss and changes in MetS status in SEQUEL subjects who had MetS at CONQUER baseline (Week 0). MetS was defined (by the US MetS criteria) as presence of ≥3 of the following 5 risk factors: waist circumference ≥102 cm in men, ≥88 cm in women; triglycerides ≥1.7 mmol/L; HDL cholesterol (HDL-C) <1.03 mmol/L in men, <1.3 mmol/L in women; systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg; and fasting glucose ≥5.6 mmol/L. **Results:** At Week 0, 59.3% (n=400) of SEQUEL subjects met the criteria for MetS (placebo, n=141; 7.5/46, n=86; 15/92, n=173) with a similar distribution of number of risk factors (3, 4, or 5) within each group at baseline. For subjects with MetS at baseline, least-squares (LS) mean percent weight loss at Week 108 was significantly greater for both doses of PHEN/TPM CR compared with placebo: 2.8%, 10.0%, and 10.8% for placebo, 7.5/46, and 15/92, respectively (intent-to-treat with last observation carried forward; P<0.0001). After 108 weeks, more subjects receiving PHEN/TPM CR no longer met the criteria for MetS than those receiving placebo: 44.7%, 51.2%, and 57.2% for placebo, 7.5/46, and 15/92, respectively (P=0.0269 for 15/92 vs placebo). Conversely, in subjects without MetS at baseline (n=274), progression to MetS was 27.9% with placebo vs 14.9% and 6.6% with 7.5/46 and 15/92, respectively (P<0.0001 for 15/92 vs placebo). At Week 108, more subjects receiving PHEN/TPM CR saw a decrease in the number of MetS component traits compared with placebo (P<0.05). Treatment with 15/92 resulted in greater improvements in most of the individual components of MetS (fasting glucose, triglycerides, HDL-C, and waist circumference when compared with placebo (P<0.05 for all comparisons). PHEN/TPM CR was generally well tolerated, with an overall completion rate of 84% on study drug. The most common treatment-emergent adverse events (upper respiratory tract infection, constipation, and paraesthesia) were similar to those experienced during CONQUER, although the overall incidence was lower in the extension.

Conclusion: In subjects with MetS at baseline, PHEN/TPM CR-treated subjects demonstrated sustained weight loss after 108 weeks that was significantly associated with resolution of the MetS diagnosis in more than half of PHEN/TPM CR-treated subjects and resulted in improvements in the individual components of MetS.

Clinical Trial Registration Number: NCT00796367

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Long-term glycaemic effects of weight loss with low-dose, controlled-release phentermine/topiramate (PHEN/TPM CR) in overweight/obese subjects with diabetesL.F. Van Gaal¹, L.J. Cheskin², T. Najarian³, W.W. Day³, C.A. Peterson³;¹Department of Endocrinology and Diabetology, Antwerp University Hospital, Edegem, Belgium, ²Department of Health Behavior & Society, Johns Hopkins Weight Management Center, Baltimore, MD, USA, ³VIVUS, Inc., Mountain View, CA, USA.

Background and aims: Obesity is a major public health problem associated with type 2 diabetes mellitus (T2DM). An investigational combination of PHEN/TPM CR demonstrated significant weight loss and improvement in glycaemic parameters in the 56-week CONQUER study involving 2487 adults with ≥ 2 weight-related comorbidities. SEQUEL, a blinded 52-week extension of CONQUER, was conducted to determine long-term safety, weight loss, and glycaemic effects of PHEN/TPM CR at 108 weeks.

Materials and methods: SEQUEL (N=675) maintained the randomization from CONQUER, with subjects receiving placebo (n=227), PHEN 7.5 mg/TPM CR 46 mg (7.5/46, n=153), or PHEN 15 mg/TPM CR 92 mg (15/92, n=295); all subjects also received lifestyle counseling. Subjects with T2DM could be treatment-naïve or receiving metformin monotherapy; they were managed per ADA standards of care, which included the potential for changes in their antidiabetic treatment regimen.

Results: Within the SEQUEL population, 145 (21.5%) subjects had T2DM at baseline. At Week 108, least-squares (LS) mean percent weight loss in the intent-to-treat population with last observation carried forward was 1.8%, 9.3%, and 10.5% for placebo, 7.5/46, and 15/92, respectively ($P<0.0001$ vs placebo). In subjects with T2DM, LS mean percent weight loss at 108 weeks was 2.0%, 9.0%, and 9.0% for placebo, 7.5/46, and 15/92, respectively ($P<0.0005$ vs placebo). Glycaemic improvements in T2DM subjects at 108 weeks were greater in the PHEN/TPM CR treatment groups, although the differences were not statistically significant (Table). In addition, a trend towards reduction of concomitant antidiabetic medications was evident in PHEN/TPM CR-treated subjects. At Week 108, 3.1% of the 15/92 group had reduced their antidiabetic medications compared with 1.3% of placebo subjects; a greater percentage of placebo-treated subjects increased their medications vs the 15/92 group (8.4% and 3.1%, respectively; $P=0.0130$ for between-group differences). Overall completion rate was 84%. Common adverse events were constipation, dry mouth, paresthesia, and dysgeusia. The pattern across treatment groups was similar to that reported in Week 56 of the CONQUER trial, although overall incidence rates were lower at 2 years.

Conclusion: Long-term (2-year) weight loss observed with PHEN/TPM CR was associated with sustained improvements in glycaemic parameters in subjects with T2DM, with treated subjects demonstrating a trend towards reduction of antidiabetic medication use. These data suggest that PHEN/TPM CR has the potential to bring about sustained weight loss, improved glycaemic status, and decreased concomitant antidiabetic medication use in obese subjects with T2DM.

Table. LS Mean Change in Glycaemic Parameters at Week 108 (T2DM Population)

	Placebo	7.5/46	15/92
HbA1c (%)	-0.04	-0.42	-0.23
Fasting glucose (mmol/L)	-0.35	-0.59	-0.41
Fasting insulin (pmol/L)	-29.52	-52.02	-43.48

Clinical Trial Registration Number: NCT00796367

Supported by: VIVUS, Inc.

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Decreased progression to diabetes in subjects with prediabetes after 2 years of treatment with controlled-release phentermine/topiramateW.T. Garvey¹, B. Troupin², W.W. Day²;¹Department of Nutritional Sciences, University of Alabama at Birmingham, ²VIVUS, Inc., Mountain View, USA.

Background and aims: Obesity can cause impaired glucose metabolism, and those obese patients who meet criteria for prediabetes, defined as impaired fasting glucose ≥ 5.55 and ≤ 6.94 mmol/L) or impaired glucose tolerance (oral

glucose tolerance test ≥ 7.77 and ≤ 11.0 mmol/L), are at a particularly high risk of developing type 2 diabetes (T2DM).

Materials and methods: The SEQUEL study assessed the long-term ability of controlled-release phentermine plus topiramate (PHEN/TPM CR) to induce and sustain weight loss and to impact weight-related comorbidities. SEQUEL was a 52-week extension in subjects completing the 56-week CONQUER study and maintained the original randomization and blinding from CONQUER (placebo, PHEN 7.5 mg/TPM CR 46 mg [7.5/46], and PHEN 15 mg/TPM CR 92 mg [15/92]). All subjects (PHEN/TPM CR and placebo) received lifestyle intervention for weight loss and were actively managed to standard of care throughout the 2 year treatment period. This prespecified subanalysis evaluated weight loss and changes in glycaemic status in SEQUEL subjects who had prediabetes at CONQUER baseline (Week 0, n=316). All analyses reflect changes from baseline (Week 0) to Week 108.

Results: After 108 weeks of treatment, least-squares (LS) mean percent weight loss in patients with prediabetes was significantly greater with both doses of PHEN/TPM CR plus lifestyle vs placebo plus lifestyle intervention ($P<0.0001$; intent-to-treat with last observation carried forward): 2.2%, 11.1%, and 12.7% for placebo (n=103), 7.5/46 (n=83), and 15/92 (n=130), respectively. More subjects with prediabetes who were treated with 15/92 achieved normoglycaemia than those receiving placebo (54.6% vs 33.0%, $P=0.0004$ vs placebo). PHEN/TPM CR 15/92 also dramatically reduced rate of progression to T2DM when compared with placebo (0.8% vs 5.8%; $P=0.0004$). Effects of 7.5/46 were not significantly different from placebo in the prediabetic subpopulation ($P=0.5930$ vs placebo), with the 15/92 dose being statistically superior to 7.5/46 on both measures ($P<0.01$ vs 7.5/46). Dose-related improvements in fasting insulin (pmol/L) at Week 108 were observed with PHEN/TPM CR when compared with placebo: LS mean change of -19.4, -34.6, and -38.2 for placebo, 7.5/46, and 15/92, respectively ($P=0.0314$ vs placebo for 15/92). PHEN/TPM CR was well tolerated, with a higher percentage of eligible CONQUER subjects on 15/92 (86%) choosing to enrol in the extension than either 7.5/46 (79%) or placebo (69%). Common adverse events over the 108 weeks were upper respiratory tract infection, constipation, dry mouth, paresthesia, and dysgeusia; the pattern was similar to that experienced at Week 56 of CONQUER, with lower overall incidence rates at 2 years.

Conclusion: In subjects with prediabetes, PHEN/TPM CR plus lifestyle intervention was associated with substantial weight loss that was maintained over 108 weeks compared to lifestyle intervention alone. PHEN/TPM CR also led to improvements in glycaemia and decreased hyperinsulinemia, and PHEN/TPM CR 15/92 markedly decreased the rate of progression to overt T2DM.

Clinical Trial Registration Number: NCT00796367

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Legacy analysis of STOP-NIDDM substudy: 9-year follow-up investigation after end of trialF. Schaper¹, C. Koehler¹, E. Henkel¹, J. Stelzer¹, R. Staudte¹, J.-L. Chiasson², H. Stein³, M. Hanefeld¹;¹Centre of clinical studies, GWT-TUD, Dresden, Germany, ²University of Montreal, Canada, ³Bayer Healthcare, Leverkusen, Germany.

Background and aims: Postprandial (pp) hyperglycemia is a major risk factor for cardiovascular disease (CVD). Acarbose, an α -glucosidase inhibitor, specifically reduces pp glucose excursion. It could be shown in the Study To Prevent Noninsulin-Dependent Diabetes Mellitus (STOP-NIDDM) that acarbose treatment in IGT reduces the risk of diabetes (DM) by 36% if the criteria of DPP were used. Furthermore in a substudy with intima media thickness (IMT) measurement we could demonstrate that control of pp hyperglycemia with acarbose was able to achieve non-progression of IMT after a follow-up time of 3.9 years. The objective of this legacy study was to determine whether administration of acarbose can delay or prevent the progression of IMT of common carotid arteries (CCA) after the end of the trial. Secondary objectives were newly diagnosed DM and hypertension.

Materials and methods: The STOP-NIDDM trial was a multinational double-blind placebo-controlled study with a total number of 1429 eligible subjects randomized to receive either 100 mg acarbose 3 times a day or placebo. Our single-center STOP-NIDDM substudy was based on 132 participants, recruited in Dresden, Germany. In the legacy investigation the patients who took part in the sub-study were examined by ultrasonography of the distal CCA as originally described by Pignoli. Patients without diagnosed DM received an OGTT with 75g glucose. Anamnestic data were collected and weight, blood pressure and HbA1c were measured.

Results: All 132 patients who completed the sub-study were contacted for the legacy visit, 8 patients were lost to follow-up and 14 rejected to participate at the legacy visit, 11 patients died (6 in Acarbose (A) group and 5 in placebo (P) treatment (n.s.)). A set of 99 patients was examined altogether in the legacy visit, 12 patients were excluded because of missing IMT data. Finally we analyzed data of 43 subjects in A and 44 in P with an average follow-up time of 9.8 yrs. At the legacy visit IMT was similar in both groups (0.81 ± 0.12 (A); 0.80 ± 0.14 mm (P)). We found an increase in HbA1c from 5.5% to 6.2% (A) and 6.0% (P), 34.4% of the patients developed DM (n.s. between the groups) after the end of the trial; and 19.5% developed hypertension. After the trial only 4 patients were treated with acarbose in the follow-up time.

Conclusion: We found no significant difference in IMT after 10 years follow-up without acarbose. Otherwise our results indicate that lower incidence of DM and hypertension at the end of the STOP-NIDDM trial was due to treatment of IGT with acarbose rather than a result of primary prevention. Patients with IGT plus IFG seen in the STOP-NIDDM trial represent a high risk group for DM and hypertension. By extrapolation of the trial and posttrial results our data suggest that such high risk patients should be considered for long term treatment with acarbose.

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PS 078 Established treatments in type 1 and type 2 diabetes

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Initial sulphonylurea monotherapy led to more frequent and earlier initiation of insulin therapy in older patients with type 2 diabetes mellitus

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Background and aims: In type 2 diabetes mellitus (T2DM), the progressive loss of β -cell function over time requires treatment intensification and eventually initiation of insulin in many patients. Relative to metformin, a greater rate of decline in β -cell function over time has been observed with sulphonylurea treatment. The present study examined the association between initial monotherapy with metformin or sulphonylurea and subsequent initiation of insulin in older patients with T2DM.

Materials and methods: In a retrospective cohort study using the GE electronic medical record database, eligible patients with T2DM included those ≥ 65 yrs who received their first prescription of sulphonylurea or metformin as initial monotherapy between 2003 and 2008. The follow-up lasted to the end of 2009 or the patient's latest data available. Insulin initiation was determined by prescription records. Logistic regression analysis evaluated the likelihood of insulin addition. Cox regression model estimated time to initiation of insulin. Differences in baseline characteristics (demographics, clinical and laboratory measures, and comorbidities) were controlled for using propensity score matching (PSM).

Results: Overall, 12,036 patients were included in the analysis with 6,018/group. Mean age was 75 yrs and 50% were male. While controlling for differences in baseline characteristics using PSM, patients who initiated with sulphonylurea had a significantly ($p < 0.001$) higher incidence of insulin addition (2.8% vs. 1.4%) compared to those initiated with metformin after 1 year of follow up. The likelihood of initiating insulin was higher in patients initiated with sulphonylurea than with metformin (odds ratio [95% CI] = 1.96 [1.51, 2.55]; $p < 0.001$). Sulphonylurea use was also significantly associated with shorter time to insulin use compared to metformin (hazard ratio [95% CI] = 2.20 [1.91, 2.52]; $p < 0.001$). The rate of insulin addition remained significantly higher ($p < 0.001$) with initial sulphonylurea monotherapy vs. metformin after 2 and 3 yrs of follow-up (6.1% vs. 2.6%, and 8.1% vs. 3.9%, respectively).

Conclusion: In a cohort of older patients with T2DM initiating antihyperglycaemic therapy, patients who started with sulphonylurea monotherapy received insulin therapy more frequently and earlier than those who started with metformin.

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Association of sulfonylurea with overall and cardiovascular mortality: a systematic review and meta-analysis

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Background and aims: Sulfonylureas (SU) are widely used for treatment of type 2 diabetes mellitus (T2DM). Recent reports have re-enforced the debate that SU treatment might be associated with an increased cardiovascular (CV) mortality in T2DM patients. We conducted a systematic review and meta-analysis to evaluate the overall and CV mortality of T2DM patients receiving SU versus any other diabetes treatment, including insulin.

Materials and methods: We performed a systematic research through electronic medical databases (MEDLINE, EMBASE) to identify any studies on SU and CV disease, and on SU and mortality, from inception through December 2010, and an additional hand search for relevant studies. Observational studies and randomised trials were included in the meta-analysis if they reported raw data on overall or CV mortality during SU treatment in T2DM patients. Randomised studies were included only if the SU treatment period lasted > 12 months. Raw data were combined using a random-effects model. Results are presented as odds ratios (ORs) and corresponding 95% confidence intervals (CIs).

Results: Of 4,828 study titles and abstracts reviewed, data from 19 studies were included in the final analysis, comprising a sample of more than 230,000 patients overall. SU single agent or combination treatment was associated with increased overall and CV mortality risk when compared to any non-SU treatment. Overall mortality, based on 18 studies, was: OR 1.39, 95% CI 1.13–1.71. CV mortality, based on 7 studies, was: OR 1.68, 95% CI 1.17–2.43. Single agent SU treatment was associated with even higher ORs for overall and CV mortality when compared to single agent metformin treatment. Overall mortality, based on 14 studies, was: OR 2.04, 95% CI 1.60–2.60. CV mortality, based on 6 studies, was: OR 2.52, 95% CI 1.75–3.63. The validity of the pooled OR estimates was limited by the high level of heterogeneity across studies ($I^2 > 90\%$), and the inherent biases and differences in study designs of the observational studies included.

Conclusion: SU treatment was associated with significant increases in overall and CV mortality. Although this meta-analysis was limited by the high heterogeneity of the studies included, patients and physicians need to consider the potential increase in mortality risk when using SU for T2DM treatment. Supported by: Lilly Deutschland GmbH, Bad Homburg, Germany

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Acarbose or nateglinide for decreasing postprandial glucose excursions: key predictive factors identified by continuous glucose monitoring (CGM)

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Background and aims: Postprandial glucose excursion contributes to the development of microvascular and macrovascular complications in any type of diabetes. Short-acting insulin secretagogue, nateglinide, and α -glucosidase inhibitor, acarbose, are commonly used for postprandial hyperglycemia in patients with type 2 diabetes. However, there is no report examining specific glucose excursions in patients treated with these antidiabetic agents and key clinical factors, which contribute to predict those effect on reduction of postprandial glucose excursions. In the present randomized clinical study, we examine the ability of acarbose and nateglinide to improve postprandial hyperglycemia by continuous glucose monitoring (CGM). We tried to identify key clinical factors based on which we can predict the effectiveness of nateglinide and acarbose to reduce postprandial hyperglycemic excursion in type 2 diabetes.

Materials and methods: We performed a randomized clinical trial assigned 11 patients with type 2 insulin-independent diabetes with postprandial hyperglycemia. CGM was performed using the CGMS-gold system (Medtronic) for three days. On the first day, glucose excursions with no medication were analyzed. The participants were then randomly assigned to two groups. In the first group, acarbose 100 mg or nateglinide 90 mg three times daily were administered on day 2 and 3, respectively, and vice versa in the second group. A standardized 460 kcal meal was used as a breakfast during the study period.

Results: The CGM data showed that the mean glucose value in the day with acarbose was lower than that with nateglinide in 4 of 11 participants. In remaining 7 participants, nateglinide exhibited a predominant effect on reducing mean glucose value. Other measures of glycemic excursions including standard deviation and AUC above 140 mg/dl of CGM measurements showed significant correlations with the mean glucose value ($p < 0.05$). Therefore, the participants could be divided into two groups: the acarbose-remediable group and nateglinide-remediable group based on the mean glucose value. Analysis of basal clinical and laboratory data including fasting blood glucose levels, HbA1c, fasting IRI, HOMA-IR, HOMA- β , serum fasting C-peptide, IRI, urinary C-peptide excretion, BMI, age, and duration of diabetes, revealed that serum fasting C-peptide was significantly different between the two groups ($p < 0.05$). The serum C-peptide level was 1.18 ± 0.18 ng/ml (mean \pm SD, 95% CI -0.89–1.47) or 2.44 ± 0.83 ng/ml (1.65–3.23) in the acarbose- or nateglinide-remediable group, respectively. A cut-off value of C-peptide set at 1.5 ng/ml can distinguish the remediable agent with 100% sensitivity and specificity.

Conclusion: In type 2 diabetes patients who maintained ability of insulin secretion (serum C-peptide ≥ 1.5 ng/ml), nateglinide exhibited a higher suppressive effect on postprandial hyperglycemia rather than acarbose. In contrast, patients with a decreased ability of insulin secretion (serum C-peptide < 1.5 ng/ml) should be treated with acarbose, since the effect of acarbose is independent of the ability of insulin secretion.

Clinical Trial Registration Number: UMIN000002995

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The improvement of endothelial function in type 1 diabetic subjects treated with metformin is independent by its glycometabolic effects

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Background and aims: Impaired endothelial function is considered an early sign of atherosclerosis in patients with type 1 diabetes (T1DM). Tight glycaemic control using intensive insulin therapy was shown to reduce rates of vascular complications in T1DM. No study had already assessed the effect of metformin, an oral biguanide glucose-lowering agent, widely used in the treatment of type 2 diabetes, on vascular function, in patients with T1DM.

Materials and methods: We enrolled 35 T1DM patients (age 36.2 ± 5.9 , 20 M) without any overt cardiovascular disease, similar for clinical and laboratory variables. Patients were randomized to receive 3x850 mg of metformin (M-group, n=20), or placebo (P-group, n=15) in addition to their standard daily therapy. Peripheral endothelial function was assessed, at baseline and at a 6-month follow up, by measuring right brachial artery dilation during post-ischemic forearm hyperemia (flow mediated dilation, FMD) and in response to administration of 25 μ g of sublingual glyceryl trinitrate (nitrate-mediated dilation, NMD).

Results: At baseline FMD and NMD were similar in the 2 groups (respectively, FMD values were: $7.7 \pm 2.7\%$ in M-group vs. $8.5 \pm 3.3\%$ in P-group, $p=0.3$; NMD values were: $10.1 \pm 2.5\%$ in M-group vs. $10.3 \pm 2.8\%$ in P-group, $p=0.7$). Compared to baseline, FMD at follow-up improved significantly in M-group patients ($8.6 \pm 2.7\%$, $p < 0.01$) but not in P-group patients ($8.5 \pm 2.9\%$, $p=0.9$). No change was observed for NMD. Moreover, glycemic variability (CONGA1-5 and standard deviation) did not change significantly in both M and P-group.

Conclusion: Metformin improves endothelial function independently by its metabolic effects. Further investigations about the potential cardiovascular-protective effects of metformin therapy in patients with T1DM are warranted.

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Changes in arterial stiffness and circulating adiponectin during one-year metformin treatment for nonalcoholic steatohepatitis: beyond glucose control improvement

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Methods and aims: Insulin resistance (IR) is the major driving force behind development and progression of atherosclerosis in patients with nonalcoholic fatty liver disease (NAFLD). Therefore, correction of IR is a relevant therapeutic target. We performed the current trial to assess whether 12-month metformin therapy improves vascular stiffness in patients with NAFLD, assess if this improvement is associated with change in glucose control, insulin level and circulating adiponectin.

Methods: In randomized, placebo controlled study, 63 patients with NAFLD were assigned to one of two groups: Group 1 received daily metformin; Group 2 received placebo. Pulse wave velocity (PWV) and augmentation index (AI) were performed using SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia) at baseline, at 4- and 12-month treatment period. Metabolic parameters, insulin resistance markers and serum adiponectin levels were determined.

Results: Among metformin treated patients: PWV and AI decreased significantly during the study. In placebo group: neither PWV nor AI improved significantly during the treatment period. Change from baseline AI as well as PWV was significantly greater in patients treated with metformin than in the placebo group ($p < 0.002$ and $p < 0.001$, respectively). The decrease from baseline HOMA-IR as well as HbA1C was marginally greater in the active treatment group compared to the placebo group. In multiple linear regression analysis, the independent predictors of arterial stiffness improvement were metformin treatment and increase in circulating adiponectin levels.

Conclusion: Metformin treatment was associated with significant decrease in PWV and AI in NAFLD patients. This beneficial vascular effect was associated with increase in circulating adiponectin levels suggesting that adiponectin is an important mediator of the insulin-sensitizing effects of metformin.

Clinical Trial Registration Number: NCT01084486

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Pharmacological characterisation of metformin-induced intestinal contraction

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Background and aims: Diarrhea is one of the side effects commonly reported with metformin treatment. The cause of diarrhea is unknown. The aims of this study were: 1. To determine the contractile response of the rat isolated ileum to metformin and characterize pharmacologically the mediating receptor. 2. To verify the possible changes of the metformin-induced intestinal contraction in diabetic (DM) rats.

Materials and methods: Ileum segments isolated from male Wistar rats were prepared for isometric contractile concentration-response (CR) curves (not cumulative) for metformin (1–36 μ M) and 5-HT (0.1–60 μ M). Frequency-response (FR) curves were obtained through electrical field stimulation (EFS). Two successive CR curves were performed, after 100 μ M acetylcholine; 15 min before each dose of the second CR curve, 10 μ M atropine, 1 μ M mepyramine, 1 μ M ketanserin, 1 μ M SB 224,289, 1 μ M ritanserin or 1 μ M methiothepin were added; 300 μ M hexamethonium, 1 μ M tetrodotoxin (TTX) and 250 μ M NG-nitro-L-arginine were added. The same procedure was performed in Goto-Kakizaki (GK) rats, a non-obese animal model of DM2. In each assay, control segments were used with the appropriate solvent of each drug. Ileum segments were also used for histological section and immunohistochemical techniques to study the immunoreactivities of 5-HT1B and 5-HT2A receptors.

Results: Metformin, in therapeutic levels (6–36 μ M), caused concentration-dependent contractions (E_{max} of 12.20 ± 0.79 mN, $n=48$; pEC_{50} of 4.89 ± 0.034 , $n=47$); 36 μ M metformin did not significantly change the rat ileum frequency-dependent contractile response to EFS suggesting a non-neuronal mechanism of action, which was confirmed by the unchanged metformin CR curve in the presence of hexamethonium and TTX. Moreover, the metformin CR curve was not significantly altered by atropine nor by NG-nitro-L-arginine, excluding the involvement of muscarinic receptors or NO, but mepyramine, an inverse agonist of the H1 histamine receptor, did significantly change the metformin-induced contraction. Concerning 5-HT receptors, ketanserin and SB 224,289 did not change the metformin-induced contraction, which means that 5-HT_{2A/2C} and 5-HT_{1B} receptors are not involved. However, methiothepin (a non-selective 5-HT receptors antagonist) and ritanserin (a non-selective 5-HT₂ antagonist), almost abolished the metformin-induced intestinal contraction which proves the involvement of 5-HT receptors, namely 5-HT_{2B} receptors. The effects of ketanserin and ritanserin on the metformin CR curve were also observed on the 5-HT CR curve. Furthermore the immunohistochemical studies did not reveal any immunoreactivity for the 5-HT_{2A} receptor on the smooth muscle layers. In GK rats the ritanserin reduction of the metformin-induced intestinal contraction only became significant at the last metformin dose of the CR curve.

Conclusion: Since in the gut about 90% of serotonin is synthesized, stored and released mainly by enterochromaffin cells, we propose that metformin may trigger the release of monoamines from enterochromaffin cells and that somehow their function is impaired in the presence of DM2.

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Pleiotropic effects of add-on therapy with pioglitazone, metformin or the combination of both in patients with type 2 diabetes on stable insulin glargine therapy

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Patients with long-term type 2 diabetes and stable insulin therapy still exhibit a high cardiovascular (CV) risk as recently shown in the DIGAMI2 study. Here we analyzed specific effects of add-on therapy with pioglitazone in comparison with metformin and their combination in type 2 diabetes patients on high CV risk under acceptable HbA_{1c} control with basal insulin glargine. In this double-blind, randomized, active comparator controlled trial 121 patients with type 2 diabetes were included. Main inclusion criteria were stable insulin treatment with basal insulin glargine, HbA_{1c} > 6.5% and < 8.5%, and

an age of 30–75 years. The patients were 63.0 (\pm 7.5) years old with BMI of 32.2 (\pm 5.3) kg/m², HbA_{1c} of 7.34 (\pm 0.53) %, insulin glargine dosage of 36.2 (\pm 20.9) units, hsCRP of 3.21 (\pm 2.54) mg/L, and Matrix Metal Proteinase (MMP-9) of 566.0 (\pm 266.2) ng/mL at baseline. Main comorbidities were hypertension in 87.6 % and CVD in 19.0 %. After a run-in period of >2 weeks with glargine monotherapy titrated to FBG < 7.8 mmol/l patients were randomized to either (1) bid 850 mg metformin, (2) bid 15 mg pioglitazone, or (3) 30 mg pioglitazone plus 1.7 g metformin for a 6-month treatment phase. Primary objective was MMP-9, secondary objectives were glucose control and specific biomarkers of inflammation and oxidative stress.

Results: Our data show that these patients despite insulin treatment exhibit an increased inflammatory activity, but normal PGF₂ α excretion.

Table 1: Course of Efficacy Parameters - Absolute Values in the Study Period

Absolute Values; Efficacy Parameter	Baseline vs. LOCF (full analysis set, n=113): arithm. means \pm std. dev. (medians); n patients'					
	1: Metformin (n=39)		2: Pioglitazone (n=37)		3: MET + PIO (n=37)	
[Unit]	Baseline	LOCF	Baseline	LOCF	Baseline	LOCF
Fasting glucose [mmol/L]	7.97 \pm 1.98 (8.10); 39	7.32 \pm 1.90** (6.79); 39	8.73 \pm 2.58 (6.79); 39	7.34 \pm 1.58** (6.99); 37	8.21 \pm 1.96 (7.81); 37	6.52 \pm 1.48** (6.33); 37
HbA _{1c} [%]	7.33 \pm 0.53 (7.30); 39	7.23 \pm 0.66 (7.10); 39	7.35 \pm 0.54 (7.20); 37	7.19 \pm 0.73 (7.20); 37	7.34 \pm 0.55 (7.30); 37	6.85 \pm 0.75** (6.70); 37
HOMA-S	3.87 \pm 3.89 ^b (2.38); 39	4.14 \pm 3.84 (2.64); 39	4.60 \pm 3.93 (2.93); 37	2.39 \pm 1.79** (1.85); 37	3.40 \pm 3.73 (1.97); 35	1.80 \pm 1.30** (1.22); 35
MMP-9 [ng/mL]	601.7 \pm 317.0 ^a (544.8); 39	651.3 \pm 365.3 (543.3); 39	535.0 \pm 214.9 (533.7); 37	480.9 \pm 232.4 (443.2); 37	581.8 \pm 260.7 (504.5); 37	514.0 \pm 219.5 (474.3); 37
hs-CRP (\leq 10) [mg/L]	3.22 \pm 2.43 (2.32); 33	2.99 \pm 2.42 (2.00); 33	3.30 \pm 2.73 (2.49); 35	2.57 \pm 2.07** (1.50); 35	2.62 \pm 1.79 (1.99); 34	1.78 \pm 1.06** (1.46); 34
Leucocytes [1/nL]	7.46 \pm 1.86 ^b (7.19); 39	7.26 \pm 1.83 (6.90); 39	7.01 \pm 1.65 (6.90); 37	6.01 \pm 1.54** (5.90); 37	6.94 \pm 1.81 (6.63); 37	6.10 \pm 1.55** (6.18); 37
NFKB [RLU]	1.248 \pm 0.756 (0.785); 38	1.228 \pm 0.688 (0.805); 38	1.024 \pm 0.630 (0.745); 36	0.992 \pm 0.588 (0.705); 36	1.172 \pm 0.707 (0.760); 35	1.154 \pm 0.703 (0.750); 35
PAI-1 [ng/mL]	71.2 \pm 23.5 (70.0); 39	61.2 \pm 27.7** (54.4); 39	71.4 \pm 25.7 (76.3); 37	62.0 \pm 29.9* (59.7); 37	70.9 \pm 27.8 (76.0); 36	53.3 \pm 30.4** (54.5); 36
E-Selectin [ng/mL]	47.1 \pm 18.7 ^a (45.3); 39	46.5 \pm 19.9 (39.2); 39	48.2 \pm 17.4 (43.4); 37	43.6 \pm 16.2** (38.7); 38	45.7 \pm 16.7 (43.8); 37	42.0 \pm 16.1** (41.2); 37
Adiponectin [mg/L]	4.43 \pm 2.61 ^b (4.16); 39	4.33 \pm 2.34 (4.00); 39	4.29 \pm 2.69 (3.85); 37	13.20 \pm 8.81** (11.49); 37	4.83 \pm 3.08 (4.11); 37	13.42 \pm 7.69** (10.94); 37
8 iso PGF ₂ α i-urine [ng/mmol]	164 \pm 89 (147); 38	186 \pm 99 (168); 38	171 \pm 131 (130); 36	186 \pm 114 (144); 36	140 \pm 46 (133); 37	162 \pm 94 (138); 37
Mean insulin con- sumpt. [units]	35.2 \pm 17.1 ^b (32.0); 38	37.7 \pm 19.6 (35.2); 38	34.5 \pm 16.9 (33.0); 35	27.2 \pm 14.6** (25.9); 35	35.4 \pm 20.3 (32.4); 37	29.4 \pm 20.9** (24.2); 37

a= $p < 0.05$ and b= $p < 0.01$ for Met vs. Pio / * = $p < 0.05$ and ** = $p < 0.01$ for within group comparisons

The addition of pioglitazone but not metformin significantly reduced MMP-9, hsCRP, E-Selectin and Leucocytes and increased insulin sensitivity and adiponectin independent from glycemic control.

The triple combination of pioglitazone with metformin resulted in better HbA_{1c} without added effect on inflammation and fibrinolysis. No serious adverse events were observed. Hypoglycemic episodes were seen in 21.4% vs. 20.0% vs. 28.2%, weight change was -0.7kg vs. +4.3kg vs. +2.7kg, and peripheral edema was observed in 11.9% vs. 40.0% vs. 20.5% in groups 1 vs. 2 vs. 3. Pioglitazone is suggested to be a rational add-on therapy to basal insulin in patients with high cardiovascular risk as it closes a gap in prevention by correction of increased inflammatory activity.

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Effects of the combination therapy of pioglitazone with insulin glargine on low density lipoprotein subfractions in patients with type 2 diabetes: the PIOcomb Study

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Background and aims: Type 2 diabetes (T2DM) is strongly associated with dyslipidemia and insulin resistance. Thiazolidinediones such as pioglitazone has been shown to improve in addition to blood glucose lowering the insulin resistance and atherogenic lipoprotein profiles. The aim of this study was to investigate the efficacy of addition of pioglitazone (PI) (vs. metformin (MI)

and vs. their combination (PMI)) to stable insulin glargine therapy in patients with T2DM on atherogenic small dense LDL particles, cholesterol particle load and particle number.

Materials and methods: Eligible patients using insulin glargine basal were randomized in this double-blind, prospective, 3-arm study and received an optimized therapy with insulin glargine plus metformin (2x850mg/day, N=39), pioglitazone (2x15mg/day, N=37) or plus the combination of both OAD (N=37) over 6 months. The LDL subfractions were isolated by very fast ultracentrifugation in VLDL, LDL1-3 and HDL. Insulin resistance was calculated by HOMA_IR model. The matrix metal proteinase 9 (MMP9) a marker for plaque stability was measured.

Results: A total of 113 (72 males, 41 females) were enrolled in the study with an average age of 63±7 years, BMI 31.9±5.2kg/m², HbA1c 7.3±0.5% and duration of diabetes 12.3±6.8 years. Overall LDL-cholesterol remained nearly unchanged by individual add on therapy. However determination of LDL-subfractions revealed distinct differences in effects on lipoprotein profile. PI treatment caused significant lowering of cholesterol concentration in atherogenic small dense LDL3 (density 1.040–1.066g/l) (change -48μmol) and increased cholesterol in HDL (+130μmol/l) after 6 months therapy while it increased with MI (+49μmol/l, n.s.). The PMI therapy decreased the cholesterol in LDL3 (change -36μmol/l, n.s.). In addition we found a significant change of cholesterol load per LDL3 particle of: -61.6mol/mol in the PI group, +86.4mol/mol in the MI group and -37.1 mol/mol in the PMI group. Vice versa we found an increase in cholesterol in LDL1 after PI therapy and a decrease in the MI group. In multivariate regression model with HOMA_IR (end of treatment) as dependent variable was found female gender, cholesterol concentration in all LDL subclasses, MMP9 and the study treatment (MI) as independent risk marker.

Conclusion: The comparison between MI vs. PI vs. PMI showed a decrease of cholesterol in small dense LDL and in LDL3 particle cholesterol load and a shift to LDL1 after 6 months PI therapy. These data suggest that in type 2 diabetic patients with high cardiovascular risk pioglitazone as add on therapy has beneficial effects on atherogenic lipoprotein profile and the insulin resistance whereas this was not achieved with metformin.

Clinical Trial Registration Number: NCT00770445

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Phenotypic and functional ex vivo characterisation of endothelial progenitor cells and effects of the PPAR-γ agonist pioglitazone

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Background and aims: Impairment in the number and function of endothelial progenitor cells (EPCs) has been implicated in the pathogenesis of cardiovascular (CV) disease in conditions characterized by an increased CV risk such as diabetes and insulin resistance. We recently demonstrated that EPC number is also reduced in subjects with subclinical insulin resistance. The PPAR-γ agonist pioglitazone, exerts beneficial CV effects that extend beyond its anti-hyperglycemic effects. Due to the lack of a standardised method for EPC identification, we first carried out a phenotypic and functional *ex vivo* EPC characterization. We subsequently investigated whether the CV beneficial effects of pioglitazone are mediated by an improvement in EPC biology independently of its metabolic action

Materials and methods: We conducted experiments for *ex vivo* isolation of two types of EPCs, namely early and late-outgrowth EPCs, obtained through different culture methods and times. Early EPCs were obtained after culturing lymphomonocytes on fibronectin-coated dishes in EGM-2 medium for 7 days. In the same culture conditions late-outgrowth EPCs were obtained after 3–4 weeks. Early and late-outgrowth EPCs were phenotypically characterized by immunofluorescence and FACS analyses. Specific endothelial proprieties such as acLDL uptake and lectin binding capacity were tested. Tubular formation assay was performed by co-plating cells on Matrigel with HUVEC to test EPC function. Pioglitazone (10μM) or PPARγ antagonist GW9662 were added to the culture media to test the effects of the drug on EPC viability/proliferation (VisionBlue-Quick cell viability assay kit), apoptosis and function.

Results: Early EPC showed the co-expression of monocyte-macrophage (CD14, CD11b, CD44) and endothelial cell-surface (CD31, KDR) markers and co-operated to tube formation. Late out-growth EPCs showed a higher expression of CD34 and of mature endothelial cell-surface markers (CD31, KDR, CD146, CD105). Late out-growth EPCs were able to form colonies and

were capable of autonomously forming tubular-like structures on Matrigel. The addition of pioglitazone improved early and late EPC proliferation by 20% and 100% respectively compared to the antagonist-containing and control cultures. No difference in apoptosis induction was observed in the different culture conditions. In the presence of pioglitazone EPCs showed a more elongated shape and the expression of adhesion (PECAM and ICAM-1) molecules was reduced. We are currently analysing data from the tubular formation assays.

Conclusion: Our data, although preliminary, suggest a putative direct beneficial effect of pioglitazone on EPC biology with potential therapeutic implications. Morphological and phenotypical changes of early-EPCs might suggest a protective effect of pioglitazone on the vascular system.

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Predicting long-term HbA_{1c} efficacy from early steady-state FPG responses: a model-based meta-analysis of anti-diabetic treatments

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Background and aims: The prediction of long-term clinical efficacy outcomes from early biomarker signals can assist efficient and accelerated drug development by identifying efficacious dose(s), terminating ineffective dosage regimen(s) in a timely manner and comparing new drugs to existing therapies. A model-based meta-analysis was implemented to establish the dose-response profiles for the two key glycaemic efficacy endpoints, glycosylated hemoglobin (HbA_{1c}) and fasting plasma glucose (FPG). Additionally, the link between the HbA_{1c} response, which takes a longer time to reach steady-state, and the FPG steady-state effect, which is achieved at much earlier times, was established.

Materials and methods: A systematic review of literature data sources from 1990 through 2010 of randomized, placebo-controlled clinical trials of anti-diabetic agents from various drug classes (GLP-1 analogues, DPP-4 inhibitors, SGLT-2 inhibitors, thiazolidinediones) administered either as mono- or combination therapies in type 2 diabetes mellitus patients was conducted for this analysis. The dose-response relationships for the change from baseline (CFB) in the steady-state FPG and HbA_{1c} data was described by separate inhibitory E_{max} models with select shared parameters using a nonlinear mixed-effects model. The FPG maximal response and the scale parameter linking the FPG and HbA_{1c} steady-state effects were estimated separately for each drug class. The drug potencies were estimated uniquely for each drug and were shared by the FPG and HbA_{1c} models. The effect of covariates including but not limited to age, disease duration, Asian subjects, body mass index, and combination therapy on the maximal reductions were also investigated.

Results: A total of 595 steady-state observations from 117 trials that met the selection criteria were analyzed by the proposed model, which predicted the dose-response relationships for the two efficacy endpoints reasonably well. The model estimated a maximal decrease in the FPG CFB of -50.2, -20.3, -34.2 and -52.5 mg/dL for the GLP-1 analogues, DPP-4 inhibitors, SGLT-2 inhibitors, and thiazolidinediones respectively. The maximal decrease in the HbA_{1c} CFB for the GLP-1 analogues, DPP-4 inhibitors, SGLT-2 inhibitors, and thiazolidinediones, was estimated to be -1.5, -0.85, -0.71 and -1.4%, respectively. The analysis indicated that subjects with higher baseline FPG and HbA_{1c} values had greater reductions from baseline in their respective responses. On average, the maximum response reductions achievable in Asian subjects were higher as compared to non-Asian diabetic patients. The model predicted that for every 1 mg/dL reduction in the placebo-subtracted FPG response to an anti-diabetic treatment, the decrease in the placebo-subtracted HbA_{1c} endpoint ranged from 0.021% to 0.042% depending on the drug class.

Conclusion: The CFB in FPG and HbA_{1c} endpoints were adequately described by the joint model. Long-term HbA_{1c} responses can be predicted from steady-state FPG measures obtained from abbreviated clinical trials. The scale parameter relating the registration endpoint, HbA_{1c}, with FPG was uniquely identified for the different anti-diabetic drug classes.

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Motivating type 1 diabetic patients to learn carbohydrate counting through a cultural/aesthetic experience

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Background and aims: Carbohydrate (CHO) counting is an important tool that allows diabetic patients greater diet flexibility and improved glycemic control. Yet learning this method is challenging and requires careful application. The purpose of this initiative is to propose a cultural/aesthetic approach to raising awareness and motivating type 1 diabetic patients to learn and apply CHO counting.

Materials and methods: During 3 guided tours at the “Pinacoteca di Brera” in Milan, Italy, type 1 diabetic patients were shown how to calculate the CHO content of food represented in paintings (Figure 1). Out of the 54 participants, 20 had previously received information on CHO counting (experienced group), and 34 had not (naïve group). A suitable route had previously been identified by selecting paintings depicting banquets, still lifes, and last suppers. A professional guide explained the historical and artistic value of the paintings, while a dietitian discussed content of the foods depicted. After the visit, the participants completed an anonymous questionnaire to evaluate the initiative and were asked to estimate the CHO content of 21 food items.

Results: 92% of participants considered the experience very interesting. 90% thought it improved their knowledge. 95%–97% reported that both nutritional and artistic aspects were presented clearly and in sufficient detail. 87% judged the combination of dietary and cultural information positively; no difference was observed between experienced and naïve participants. Importantly, in the naïve group 87.8% agreed to follow a training course on CHO counting, 73% claimed they would be willing to make changes in their diet and 9.7% reported that they would ask their physicians for their own insulin/CHO ratio. All participants would recommend this experience. When tested on the CHO content of 21 food items, correct answers were 34.4% and 49.6% in the naïve and experienced groups, respectively.

Conclusion: Introducing CHO counting in the context of a cultural/aesthetic experience motivated type 1 diabetic patients to learn about this method. The use of visual and verbal communication to convey the necessary dietary concepts appears to be an effective educational tool. The less formal interaction between learners (patients) and teachers (diet and art experts) away from the medical setting reportedly creates a stimulating pedagogical experience. In addition, participation in this initiative in an environment of high cultural prestige may have promoted a dynamic of “narcissistic investment” that motivates patients to engage in learning CHO counting.

Fig 1. The greengrocer: Vincenzo Campi (1536–1591). CHO content of vegetables and fruits.



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Non-adherence to lifestyle modification and its determinants among type 2 diabetic patients

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Background and aims: Non-adherence to preventive and therapeutic lifestyle recommendations among patients with diabetes is special challenge in the management of these patients. Understanding the determinants of non-adherence to lifestyle changes can help to plan and implement more intensive interventions to assist patients' long-term task of achieving beneficial lifestyle changes. This study aimed to measure the proportion of non-adherence and its determinants to lifestyle modification (diet and exercise) among a group of Bangladeshi type 2 diabetic patients.

Materials and methods: Under an analytical cross-sectional design 374 type 2 diabetic patients (age >20 years), diagnosed for at least 1 year, were purposively selected from different health care centers operated by the Diabetic Association of Bangladesh (DAB). Data were collected by a pre-tested, interviewer-administered questionnaire. Three-point scale (yes, no, sometimes) were used to assess patient adherence to lifestyle measures (diet and exercise). Patients were considered compliant if patients had adhered to a recommended dietary chart, maintain specific time of food intake and followed advised quantity and quality of food. Exercise were considered adhered if they did exercise >30 min/day. Self management was assessed by self monitoring of blood glucose, foot care and smoking practice. Anthropometric measurements were done by using appropriate tools and all biochemical data were collected from record book.

Results: Of the respondents 58% were females. The mean±SD age was 51 (±11.3) years, about 35% were aged between 40–59 years, 46% had completed high school with mean monthly income US\$ 398 (±375) and 75% lived in urban areas. Mean BMI was 25.7 (±3.6) Kg/m² and about 69% were overweight or obese according to Asian BMI cut-off value. Mean fasting serum glucose was 8.4 (±3.4) mmol/l and about 58% patients' HbA1c level was >7%. About 60% patients attended diabetes education class at least once followed by 24% never attended. Non-adherence rate of diet was 88% and exercise was 25% -overall 89% (95% CI 87.4–91.0) had non-adhered to both diet and exercise. About 32% patients non-adhered to self blood glucose monitoring, 70% to foot care and 6% had smoking habits. The main barriers to adherence to blood glucose monitoring was that they did not believe it is useful (65%) and barriers to do exercise were always being busy (44%) and co-existing diseases (9%). Association was found between non-adherence of diet and residence and nonattendance to diabetes education classes (p<0.05). Age, gender and nonattendance to diabetes education classes were associated with non-adherence to exercise (p<0.05). Binary logistic regression suggests that level of education (p=0.03) and nonattendance to diabetes education classes (p=0.05) are correlates of non-adherence of diet, and gender (p=0.04), family history of diabetes (p=0.04) and age (p=0.04) are correlates to non-adherence to exercise.

Conclusion: Although majority of the patients do not follow dietary and foot care recommendations, the adherence to exercise and blood glucose monitoring is comparatively high. Diabetes education and sociodemographic factors need to be considered to improve adherence to lifestyle modification and self care.

Supported by: DAB

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The effects of physical activity in Chinese adults with type 2 diabetes mellitus: a meta-analysis

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Background and aims: Physical activity is well known as a cornerstone in the treatment of diabetes mellitus. However, the effects of regular physical activity in Chinese adults with Type 2 diabetes mellitus are unclear. Our study aims to conduct a meta-analysis of randomized-controlled trials (RCTs) in order to assess the effects of physical activity on glycemic control, lipids and lipoproteins in Chinese adults with Type 2 diabetes mellitus.

Materials and methods: Search strategy: Chinese databases: VIP (1989 to January 2011), CNKI (1994 to January 2011), CBM (1978 to January 2011),

CPCD (2000 to January 2011), and Papers on Academic Conference of China (2000 to January 2011) were searched. And also the reference lists of all articles collected were checked to ensure that no relevant suitable studies were missed. Selection criteria: All RCTs that evaluated the effects of regular physical activity (duration ≥ 12 weeks) combined with usual treatment versus usual treatment alone in Chinese adults with type 2 diabetes were included. Data collection and analysis: Two authors independently selected trials, assessed trial quality and extracted data. Continuous data were calculated as mean differences (MD). Random-effects model and fixed-effects model were used to perform meta-analysis for with and without heterogeneity respectively.

Results: Only fourteen RCTs were available for pooling involving 1025 participants. Compared with the control, the physical activity intervention significantly improved glycemic control as indicated by a decrease in glycated haemoglobin (HbA_{1c}) levels of 1.34% (-1.34% HbA_{1c}, 95% confidence interval (CI) -1.91 to -0.77; $P < 0.00001$). And there was a reduction in triglycerides (TG) levels and total cholesterol (TC) levels by 0.25mmol/L (-0.25mmol/L, 95% CI -0.36 to -0.15) and 0.74mmol/L (-0.74mmol/L, 95% CI -1.05 to -0.43) respectively with physical activity intervention. Also high-density lipoprotein cholesterol (HDL-C) levels increased by 0.12mmol/L (0.12mmol/L, 95% CI 0.08 to 0.16) and low-density lipoprotein cholesterol (LDL-C) levels decreased by 0.61mmol/L (-0.61mmol/L, 95% CI -0.82 to -0.40).

Conclusion: Although our overall results suggest that physical activity decreases HbA_{1c}, TG, TC and LDL-C and increases HDL-C in Chinese adults with Type 2 diabetes, additional randomized-controlled trials are needed on these topics.

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Improving health numeracy: utilizing objective parameters to improve shared decision making for insulin initiation

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Background and aims: Health numeracy is the ability to understand, and act upon quantitative data, in an appropriate and effective manner. Health numeracy is distinct from health literacy. Health numeracy is associated with better self-management. This study was performed at two endocrine centres in north India, to assess and improve the health numeracy of patients with type 2 diabetes, with an aim to improving the quality of shared decision making (SDM).

Materials and methods: A three months long campaign was begun to explain the meaning of HbA_{1c}, vibration perception threshold and mean plasma glucose to 1000 patients presenting with T2DM in the OPD. This study was carried out by laboratory technologists, by means of semi-structured training, at each OPD visit.

Results: At baseline, 24.5, 11.8 and 45.0 % of patients understood the meaning of HbA_{1c}, mean vibration perception threshold (VPT) and mean plasma glucose (MPG) respectively. At three months, these proportions had increased to 56.6, 90.0 and 67.3 % respectively. During these three months, 351 patients were initiated on various regimes of insulin, of which 250 consented to fill a pre structured questionnaire. 60.0 % of them felt that understanding their HbA_{1c} value had played a major role in motivating them to begin insulin. Similar response was given by 80.0 % for mean VPT and 100 % for MPG.

Conclusion: This paper highlights the efficacy of improving health numeracy as a means of enhancing shared decision making, and facilitating insulin initiation in type 2 diabetes. It reveals the higher importance of mean plasma glucose values, rather than HbA_{1c} and mean VPT in motivating patients to accept insulin.

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Continued smoking exacerbates but cessation ameliorates progression of early type 2 diabetic complications

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Background and aims: Diabetes is considered among the target groups for smoking cessation treatment, due to the increased health risks associated with smoking. The effect of 4-year smoking cessation upon type 2 diabetes (T2DM) diagnosis was assessed in this study.

Materials and methods: From 500 smokers, 200 (100 men/ 100 women: age 56.4 \pm 7.8 years) agreed to participate and were educated to smoking cessa-

tion, diet and exercise. Pharmacological interventions, smoking duration, daily frequency, and cumulative smoking exposure did not differ between the studied groups. Demographic, clinical and biochemical parameters were obtained at baseline and at 12-months follow-up. Peripheral vascular disease (PVD) was diagnosed ultrasonographically and peripheral neuropathy (PN) on neuropathy symptom and disability score. Retinopathy was diagnosed by direct funduscopy. At the end of the study, smoking status was confirmed by an expired CO level of <10 ppm.

Results: A total of 120 subjects (24%) quit smoking. Smoking cessation was accompanied by an increase in BMI ($P=0.05$), in physical activity ($P=0.01$), better glycemic ($P=0.04$), blood pressure ($P=0.03$) and lipids control ($P=0.02$) compared to smoking continuance. Smoking cessation ameliorated prevalence of PVD (8.2% vs. 7.5%, $P=0.03$), PN (15% vs. 10.9%, $P=0.04$), and retinopathy (4.3% vs. 3.9, $P=0.04$) compared with smoking continuation. Microalbuminuria was reduced at 4-year to 12.5% of the subjects who continued smoking and to 82.6% of those who quit smoking ($P < 0.001$). Multivariate logistic regression analysis demonstrated that independent predictors of the reduction in microvascular complications were amelioration of insulin-resistance [HR, 95%CI: 1.18 (1.08-1.23)], blood pressure [1.03 (1.01-1.06)], glycemic control [1.10 (1.04-1.13)] and lipids profile [1.12 (1.14-1.26)]. From treatment and lifestyle interventions, smoking cessation had the highest contribution to the reduction of microvascular complications [1.20 (1.18-1.23)]. **Conclusion:** Smoking cessation in newly-diagnosed T2DM improves insulin-resistance, glycemic control, blood pressure, and lipids profile and reduces microvascular complications. Stricter counselling about the importance of quitting smoking is necessary upon T2DM diagnosis.

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What do patients need? The development of a patient-centred health educational model for patient education

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Background and aims: Patient education is a crucial element in the treatment and care of patients with type 2 diabetes and other chronic illness. However, a majority of models for patient education is based on a professional or theoretical understanding of patients' needs for learning rather than on a patient perspective. Patient education programmes thereby risk neglecting themes and issues that are important to the patients. This might limit the effect of the patient education. The aim of the present study was to explore the educational needs from a patient perspective in order to develop a model for patient education based on needs and perspectives as defined by patients.

Materials and methods: Guided by an Action Research approach we explored the perceptions, views and experiences of patient education among patients with type 2 diabetes, COLD and heart disease, eligible to or participating in group-based patient education in four Danish municipalities. Four highly interactive workshops were conducted with 25 adult patients in August and September 2010. At the workshops customised tools were used in order to generate a dialogue about specific themes. The workshops were video recorded and the analysis was based on the constant comparative method. The insights from the workshops were presented to a group of 25 health care professionals who were then asked to translate the insights into patients' needs for patient education. The identified needs were categorised and grouped repeatedly until four basic needs or focal points for patient education emerged.

Results: The patient-based model for patient education is formed by the following four focal points:

1. Entirety is about ensuring the link to the entire life of the patient:

- Connecting the past, present and future
- Seeing the person instead of just a disease
- Ensuring a main thread in the teaching

"It's about what your life is and what keeps you going. You cannot talk illness all the time. That will make you ill" quote from a patient

2. Clarity is about creating clarity about challenges and possibilities:

- Encouraging the articulation of imbalances
- Communicating relevant knowledge
- Using visual and tangible methods

"The most important was that I realized that I cannot deal with this myself. I need help and support" quote from a patient

3. Timing is about choosing the right thing at the right time:

- Identifying the individual's own process of change
- Creating time and space for anchoring
- Pinpointing optimal moments and content

"I would like to have more time to unplanned discussions and to talk with the others" quote from a patient (about the patient education process)

4. Connectedness is about supporting the need to be social and connected:

- Strengthening group dynamics
- Encouraging the sharing of experiences
- Creating a setting that enhances confidence
- Supporting relations to surroundings

"It is really good to talk to the person sitting next to you and to the group. It gives inspiration and ideas" quote from a patient

Conclusion: In order to meet the needs of patients the developed model suggests that four focal points are central. The model can be used as a planning tool for patient education programmes as well as an analytical tool for the evaluation and quality assurance of the delivered education. Future implementation and effect studies are needed to assess the effect of the model and to assure and determine the quality of this patient-centred approach.

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Low physical activity reduces mortality in community dwelling older adults with diabetes but not in those without diabetes

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Background and aims: Most physical activity (PA) guidelines recommend at least 500 MET-minutes/week of PA for adults to achieve substantial health benefits. However, PA level may be reduced in older adults in general, and in older adults with serious comorbidities such as diabetes mellitus (DM) in particular. We used the public-use copy of the Cardiovascular Health Study (CHS) data obtained from the National Heart, Lung, and Blood Institute (NHLBI) to examine associations of various levels of PA in community-dwelling older adults with and without DM.

Methods: Of 5775 CHS participants with data on baseline leisure-time kilocalories of energy expended/week, 941 (16%) had DM. Participants were categorized into 4 PA groups (based on MET-minutes/week): Inactive (0 MET-minutes/week; n=539); low PA (0 to 499 MET-minutes/week; n=1555); medium PA (500 to 999 MET-minutes/week; n=1129); and high PA (≥ 1000 MET-minutes/week; n=2552). Cox-regression models were used to examine the relationships between PA categories and all-cause mortality during 13 years of follow-up, separately among those with and without DM.

Results: Patients (n=5775) had a mean (\pm SD) age of 73 (± 6) years, 58% were women, and 16% were African Americans. Compared with inactive older adults, medium and high PA was associated with reduced mortality regardless of DM (Table). However, only in those with DM, but not in those without DM (p for interaction, 0.002), a low PA was associated with reduced mortality (Table).

Table. Physical activity levels and mortality in older adults with or without diabetes mellitus

	Physical activity	MET-minutes per week	Mortality, %	Unadjusted hazard ratio (95% CI)	Adjusted* hazard ratio (95% CI)
No diabetes (n=4834)	Inactive	0	57%	1 (Reference)	1 (Reference)
	Low	0-499	48%	0.77 (0.66-0.89); p<0.001	1.03 (0.88-1.20); p=0.317
	Medium	500-999	41%	0.60 (0.51-0.71); p<0.001	0.85 (0.72-0.998); p=0.058
	High	≥ 1000	38%	0.51 (0.44-0.58); p<0.001	0.72 (0.62-0.84); p<0.001
Diabetes (n=941)	Inactive	0	73%	1 (Reference)	1 (Reference)
	Low	0-499	59%	0.67 (0.52-0.86); p=0.002	0.70 (0.54-0.91); p=0.007
	Medium	500-999	62%	0.67 (0.51-0.89); p=0.005	0.75 (0.56-1.00); p=0.051
	High	≥ 1000	57%	0.57 (0.45-0.74); p<0.001	0.65 (0.50-0.85); p=0.002

* Adjusted for age, gender, race, pack-years of smoking, weekly alcohol consumption, depression score, mini-mental status score, body mass index, hypertension, coronary artery disease, heart failure, atrial fibrillation, stroke, serum creatinine, left ventricular hypertrophy by electrocardiography, and left ventricular systolic dysfunction by echocardiography

Conclusion: Recommended levels of medium and high PA is associated with improved survival in older adults with and without DM. However, older adults with DM also benefit from lower levels of PA, in whom the mortality benefit does not appear to increase with higher levels of PA.

Supported by: NHLBI

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Seasonal variation of hypoglycaemic episodes in a single university hospital in Korea

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Background and aims: Hypoglycemia is a leading limiting factor for the glycemic management in diabetic patients. The main cause of hypoglycemia is insulin excess relative to carbohydrate intake. We investigated the risk factors and seasonal variation of hypoglycemic episodes in diabetic patients visiting emergency center.

Materials and methods: We selected the patients who visited emergency center in Chuncheon Sacred Heart Hospital, Hallym University for the management of hypoglycemia from January 2008 to December 2010. Hypoglycemia was defined by the symptom and serum glucose < 70 mg/dL. We excluded the patients who had no history of diabetes. We reviewed medical records in terms of age, visiting date, HbA1c (%), cause of hypoglycemia (change of food intake and physical activity), medications (sulfonylurea, glinide, or insulin), hemoglobin (g/dL), albumin (g/dL), creatinine clearance (estimated GFR by the MDRD equation).

Results: During the study period, there were 196 hypoglycemic episodes in 168 diabetic patients. The mean age was 70.3 ± 11.2 years (34-91). The level of mean glucose was 36.2 ± 12.5 mg/dL. The proportion of HbA1c $< 6.5\%$ was 50.5%. The hypoglycemic episodes was most common in summer season (June to August: 34.7%) and least common in fall (September to November: 17.9%). The main cause of hypoglycemia was decreased food intake (51%). Most patients had gastrointestinal symptoms, such as nausea, vomiting, or diarrhea. Sulfonylurea was taken by 52.6% of patients. The proportion of chronic renal failure (eGFR < 60 mL/min/1.73 m²) was 55.6%, anemia was 64.1%, and low albumin (< 4 g/dL) was 53.1%.

Conclusion: The risk factors of hypoglycemic episodes in this study were old age, good glycemic control, comorbidity (chronic renal failure, anemia, and malnutrition), summer season, and decreased food intake. The hot and wet weather in the summer in Korea might be decreased food intake, especially in old, comorbid diabetic patients. The decreased food intake in summer season should be considered for preventing hypoglycemia, especially in old, comorbid diabetic patients.

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Patients with type 2 diabetes prefer education based on participation and development of competences over information

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Background and aims: Little is known about patient preferences for diabetes patient education. The objectives of this study were to determine the preferences of patients with type 2 diabetes for format and contents of patient education and support. We investigated how patients value information, participation and competence development, and involvement of the social network. Patients were also asked to value group based versus individual education.

Materials and methods: A questionnaire was developed including questions on socio demographic data, height and weight, perceived care, psycho-social problems, social network, self-management behaviours and HbA1c-level as well as choice games concerning patients' preferences for patient education and support. Questionnaires were sent to patients with type 2 diabetes from two different populations: 1) a population of patients from a specialist diabetes clinic (n=1081, response rate 54%) and 2) a population of type

2 diabetes patients derived from a web panel consisting of a representative sample of the Danish population ($n=1461$). In total a population of 2542. The data collection took place September to November 2010. The choice game answers were analysed using the conditional logit model. Willingness To Pay for the attribute levels was calculated by dividing the estimated coefficients, β , for each attribute by the coefficient of payment. For deriving the confidence intervals for the Willingness To Pay results, 10,000 iterations were carried out by bootstrapping. Analyses were stratified for age, gender, educational attainment and other subgroups. A 5% level of significance was used. **Results:** All included attributes were significant predictors of choice ($p<0.01$) and all parameters had a positive value. Patients consistently valued acquiring competency in the included topics more than receiving information about them except for psychological problems. The difference in valuation between becoming competent and acquiring information was largest for living a fulfilling life with diabetes (willingness to pay was 92% higher for competency). Becoming able to adjust diets and exercise habits and becoming able to prevent complications were valued 35% and 46% higher, respectively, than only being informed about these topics. Patients valued support to ask questions to health care professionals nearly twice as high (€212) as receiving support to find networks (€117). Patients preferred to be involved in the planning of their diabetes care (€130) compared to planning their diabetes care alone. Patients were willing to pay €199 to be educated individually compared to education in a group of 12. Patients valued individually tailored content as worth €146 compared to prescheduled content. The ranking of the attributes and levels were similar for subgroups. Women generally had a higher valuation of attributes. Patients <60 years had a higher Willingness To Pay for all attributes and levels than older patients. Patients with HbA1c <7% exhibited higher Willingness To Pay for all attributes and levels. **Conclusion:** Patients with type 2 diabetes significantly value participation in patient education, development of competencies for prevention of complications and support from the social network in disease management. Patients prefer an individually targeted approach.

by educators played a significant role to increase awareness ($\%$, 68.8 ± 10.6 vs 57.0 ± 12.8 ; $p<0.0001$) in type 2 diabetic subjects. The awareness score was found significantly lower in women than in men ($\%$, 70.6 ± 10.9 vs 66.6 ± 10.8 ; $p<0.0001$). About 76% subjects believed that taking OHA or diet for controlling diabetes is comparatively risk-free for developing complications than those who took insulin. About 47% were aware of the importance of maintaining good glycemic control and 66% considered this as a very difficult task. **Conclusion:** Bangladeshi type 2 DM subjects are moderately aware about diabetes and risk factors of NCDs. These subjects have poor level of awareness regarding risk factors of diabetes, cause of dyslipidemia and obesity, normal level of lipid profile and blood pressure. Sex, family history, acquisition of information through educational tools are the most important determinants of awareness.

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Awareness among Bangladeshi type 2 diabetic subjects regarding diabetes and risk factors of non-communicable diseases

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Background and aims: Awareness among type 2 diabetic subjects is a major determinant for the prevention of diabetes and its complications as well as related risk factors of non-communicable diseases (NCDs). This is also required to design strategy for awareness campaign. The present study aimed to assess the awareness of diabetes and the risk factors of NCDs among Bangladeshi type 2 diabetes subjects.

Materials and methods: A total of 500 subjects (43% male and 57% female; age 52 ± 11 years, mean \pm SD) were selected from different health care centers in Bangladesh under a cross-sectional design. A pre-structured interviewer administered questionnaire was used for collecting data. Questionnaire was divided into 5 main sections, namely general definition of diabetes, dyslipidemia, hypertension and obesity; risk factors of NCDs and complications, treatment and management of the diabetes. On the basis of predefined scoring the levels of awareness score was categorized as good (>75%), moderate (50–75%) and unacceptable (<50%). According to WHO (2006), BMI was classified as underweight (<18.5 kg/m²), normal (18.5–25 kg/m²), over weight (25–30 kg/m²) and obese (>30 kg/m²).

Results: The proportion of good, moderate and unacceptable level of awareness among the subjects were 31%, 62% and 7% respectively. Majority (89%) of the participants had correct understanding regarding basic definition of diabetes though 77% among them did not know about the risk factors of DM and 19% thought excess sugar intake is a major cause for developing diabetes. Only 29% were aware about the consequences of uncontrolled diabetes. Most of the participants (99%) did not have any idea about the risk factors of NCDs such as dyslipidemia, obesity and also did not know the cut-off values of BMI (100%), triglycerides (95%) and total cholesterol (82%). However, 94% of the subjects knew about hypertension and the cut-off value of blood pressure. Only 12% correctly answered regarding the causes of hypertension. Subjects (33.5%) with positive family history of DM were more aware regarding diabetes than those (26.8%) without such family history. Regarding NCDs risk factors, only 16.1% subjects with history of NCDs had good awareness. Acquisition of information through educational tools and group discussion

PS 080 Education

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Community health workers effectiveness in diabetes education

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Background: Community health workers (CHWs) are members of the community hired by the government to carry out functions related to health promotion and prevention within their communities. The use of CHW has been identified as one strategy to address the growing shortage of health workers, particularly in low-income countries. In Brazil, CHW are widely used to provide education and care for patients for a broad range of health issues, including diabetes mellitus (DM). However, few CHW are trained for diabetes education and little is known about the effectiveness of their interventions. The aim of the study is to evaluate the effectiveness of a diabetes education program delivered to CHW in improving the metabolic control of patients with type 2 diabetes.

Materials and methods: A randomized controlled trial was conducted in a primary care unit. Eight CHWs, providing care for 118 patients, were randomized in two groups to receive a one-month (four sessions, two hours each) diabetes education program (intervention group, patients $n=62$) or an education course in other health issues (control group, patients $n=56$). Each CHW were then responsible for transmitting the acquired knowledge to the patients from their respective work areas. The primary outcome was change in A1C, and the secondary outcomes were the effect in weight, blood pressure, lipids and acquired learning 4-month after the intervention.

Results: Participants mean age was 61 ± 11 years with median diabetes duration of 6 (IQR= 3 - 5) years, 35% were men and 62% were whites. Included subjects had a mean of 5.0 ± 3.5 years of formal literacy and 41.6% had a mean family income less than US\$ 450 per month. Mean BMI was 31 ± 6 kg/m² and mean A1c was $9.0 \pm 2.5\%$ (no differences between groups regarding these variables at baseline). No change was observed in patient's DM education scores (intervention: 15 ± 5.2 vs 15 ± 6.0 and control group: 14 ± 4.7 vs 15 ± 5.5 , $p=0.43$). A1c levels were reduced in both groups (intervention 9.1 ± 2.2 vs 7.5% and control 9.2 ± 2.1 vs $7.9 \pm 2.1\%$, $p<0.001$), but no statistically significant differences were observed between groups ($p=0.22$).

Conclusion: A diabetes education program centered in patient's self-management delivered for CHW did not improve the glycemic control of patients with type 2 diabetes after a 4-month period. Other DM educational strategies should be studied in order to improved diabetes control in the public health scenario.

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A diabetes specialist care inpatient team as diabetes educators

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Background and aims: To improve the education of patients with diabetes and health care professionals in a general hospital through the work of a diabetes specialist care inpatient team.

Materials and methods: A diabetes specialist care inpatient team was set up in 2006 in a 400 bed general hospital consisting of Consultant Physician, Diabetes Nurse Specialist and doctors in training. Each week the team will visit every medical and surgical ward on two days, seeing all the inpatients with diabetes either as primary diagnosis or comorbidity, and liaise with ward medical and nursing staff (HCPs).

Results: In the 6 months January to June 2008 and 2009 respectively: 367 and 397 patients were seen; 851 and 992 visits were made; 620 and 300 interventions were for direct patient advice, and 49 and 297 HCP advice; and 296 and 347 treatment changes were made. In the 2009 National Diabetes Inpatient Audit (Diabetes UK) the hospital had only 5% (insulin) prescription errors (v 19% nationally, $p<0.05$, t test) and 3% (patient) management errors (v 14%, $p<0.05$), with 100% appropriate blood glucose monitoring (v 86%). Mean number of "good diabetes days" was 5.5 v 4.2 nationally ($p<0.05$) and 83% patients (v 73%) reported a positive experience of diabetes care.

Conclusion: A diabetes specialist care inpatient team improves the quality of care and education of patients and health care professionals as evidenced by: the low frequency of insulin prescribing and management errors; the 100% appropriate blood glucose testing; and more "good diabetes days" and frequency of visits to patients with a positive outcome.

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The mode of diabetes education is a determinant of glucose control in a Chinese diabetic patient cohort

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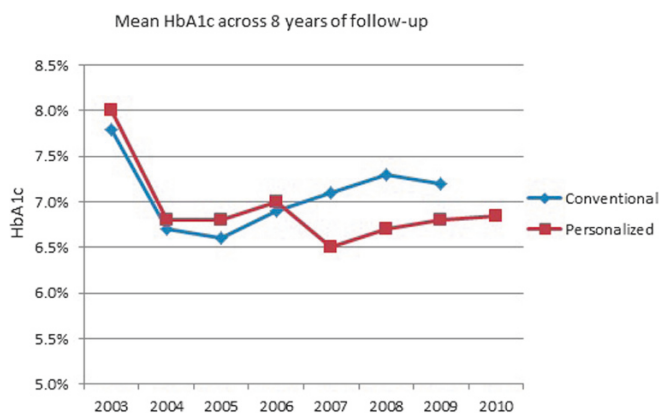
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Background and aims: Diabetes education plays an important role in patient management and metabolic control. The aim of this study was to investigate the mode of diabetes education in relation to glucose control in a Chinese cohort of diabetic patients.

Materials and methods: This retrospective study was based on a diabetes education programme (once a week) initiated in 2003 that included 156 diabetic patients (17 type 1 and 139 type 2, mean age 50 ± 5 years) from Qingdao, China. A conventional education mode was given in the way of classroom lecture to all patients from 2003 to 2006. Since 2007, personalized face-to-face group sessions (4 patients per group) was introduced due to the withdrawal from conventional education mode. The patients were classified into group 1 (conventional) and group 2 (shifted from conventional to personalized mode) during 8-year follow-up. General linear model was used to compare the HbA1c levels between groups adjusting for age, medication treatment and baseline glucose levels.

Results: There was 79 in group 1 and 77 in group 2 in 2003. 75 withdrew from group 1 and 29 withdrew from group 2 by the end of 2010. Mean HbA1c significantly decreased in both groups in 2004 ($p<0.05$) and tended to increase in 2005 and 2006. Patients in group 2 had significantly lower HbA1c in 2007 (6.5% vs. 7.1%, $p<0.05$), 2008 (6.7% vs. 7.3%, $p<0.05$) and 2009 (6.9% vs. 7.2%, $p=0.06$). We were not able to analyse the data in 2010 due to a high withdrawal rate from group 1 (Figure 1).

Conclusion: Conventional education mode is not enough for long-term ideal glucose control. Regular improvement of education mode might be an important way to enhance patients' interests of participating in an educational programme and improve glucose control.



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Beyond the "self" in diabetes care: what are the tools and what are the effects?

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Background and aims: Living with diabetes has serious implications for everyday life and social relationships, especially in relation to social activities such as diet, exercise and clinical care. Programmes of patient education are dominated by an individualistic approach to diabetes management, emphasising knowledge, individual motivation, self-efficacy and choice. While such foci are relevant for engaging individuals in self-care, it is important to supplement such foci with approaches relating to the social formation of every-

day life and social relationships outside the formal healthcare setting in order to support long term self-care. This review aims at furthering the knowledge on how to effectively design and implement interventions targeting adult diabetic patients, that involves the social context of the diabetes patient.

Materials and methods: A systematic search was conducted in relevant databases identifying reviews published between 2000 - February 2011. Specific keywords were selected with a main focus on terms related to; diabetes, intervention and social context. 518 results were found and 19 reviews were selected after systematically reading through abstracts and titles. Inclusion criteria were: Reviews over intervention studies targeting adult diabetic patients involving other subjects than the patient or health care professional.

Results: Two kinds of interventions were identified 1) Family interventions directed at the existing social network of the diabetic patient 2) Interventions directed at peers, seeking to support through a created social network. Under both kinds of interventions we have an additional focus on studies targeting specific cultural and marginalised groups that takes social context as a basic condition of the intervention. Only two intervention studies directed at family members can be identified. These studies suggest that family interventions may be supportive as well as constraining on self-care behaviour (diet) and that the social dynamic in the family plays a significant role. Moreover, they suggest that gender makes a difference in perceived family support. There is a strong immediate positive effect on face-to-face interventions involving peers on glycemic control, quality of life and self-efficacy. There are promising preliminary results of use of peers as mentors and community health workers on clinical, psychological and behaviour outcome and are especially relevant for interventions targeting minority groups. No intervention studies on the use of peers in telephone interventions have been conducted, but a pilot study found high levels of participation and satisfaction. Adding peer support components to internet based interventions can increase their effectiveness in terms of weight loss, coping and self-efficacy. We need more knowledge about recruitment, education and support of peers.

Conclusion: This review supports the hypothesis that involving the patients' social context in interventions affects patient self-care. The review shows promising preliminary results, but more research is needed on designing, implementing and evaluating different models of interventions, especially family interventions for adult diabetic patients and the use of peers outside the clinic. Also we need more knowledge about how to create more flexible forms of interventions, where elements can be put together and adapted to family situation of the individual, life circumstances and culture.

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Effects of a newly developed education programme (MEDIAS 2 ICT) for patients with type 2 diabetes with intensive insulin therapy: results of a randomised prospective trial

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Background and aims: Introduction: In a randomized, multi-centre trial, the effect of an education programme (MEDIAS ICT) involving intensive insulin treatment for type 2 diabetic patients was compared with an established education programme as an active comparator condition (ACC). Participants of the experimental group received a group training using MEDIAS 2 ICT which comprises 10 lessons. The participants of the control group (ACC) participated in the previously established "Education programme for type 2 diabetic patients injecting prandial insulin" and the "hypertension education programme". But programmes together had duration of 10 lessons, which also consisted of 10 lessons.

Materials and methods: A total of 185 type 2 diabetic patients from 18 different study centres were randomized to either MEDIAS ICT or ACC. It was expected that MEDIAS 2 ICT would be at least as effective as the ACC with regard to improvement of HbA1c values of the participants (non inferiority hypothesis). Secondary outcome variables were the perceived burden of diabetic illness, knowledge about diabetes, Quality of Life, self management behaviour as well as the amount of lipids and blood pressure

Results: The mean HbA1c decrease was 0.37 % (from $8.2 \pm 1.1\%$ to $7.8 \pm 1.5\%$) in the ACC and 0.63 % (from $8.5 \pm 1.6\%$ to $7.9 \pm 1.2\%$) in MEDIAS ICT. The mean difference between both groups was -0.26 in favour of MEDIAS ICT, with a 95%CI ranging from -0.64 percentage points to 0.12 percentage points; this result was within the predefined limit of 0.4 percentage points for non-inferiority. Diabetes-related distress was significantly more reduced in MEDIAS ICT (-3.4 ± 7.1) than in ACC (-0.4 ± 9.0 ; $p = .031$). Both groups increased their diabetes knowledge significantly. MEDIAS ICT participants showed

a significant within group difference regarding self-care behaviour and the physical composite score of the Short Form Health Survey (SF-12), whereas ACC participants did not, but between group differences were not significant. There was no specific effect of either intervention on lipids or blood pressure.

Conclusion: MEDIAS ICT is as effective in lowering HbA1c as previously established education programmes. In addition, MEDIAS ICT has proven its efficacy regarding the improvement of diabetes knowledge, health-related quality of life, and self-care behaviour. Participants of MEDIAS ICT were able to reduce diabetes-related distress to a significantly greater extent than patients who took part in more-established traditional education programmes focusing on diabetes knowledge and adherence. Blood pressure and lipids were at an adequate level at the start of the study which did not leave much room for improvement. In sum, MEDIAS 2 ICT is an effective alternative to already established programmes used for training type 2 diabetes patients in the need for an intensified insulin therapy. We conclude that MEDIAS ICT, which involves intensive insulin treatment, provides an alternative to previously established education programmes

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Care for the elderly with diabetes

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Background and aims: Education is crucial for the control of chronic diseases. Being recognized the need for training of caregivers of people with diabetes. The project "Care for the Elderly with Diabetes" was developed to solve this problem with the aim to improve the trainees' skills in providing care to the elderly with diabetes.

Materials and methods: We conducted six training sessions, between 30/04 and 25/06/2010 for a total of 86 trainees. The topics covered were diabetes treatment, diet, exercise, metabolic control, treatment of hypoglycemia and care for the feet. To assess sensitivity to change, a questionnaire was administered to all subjects before, at the end and after training and subsequently analyzed the mean differences in knowledge, using t test for paired samples.

Results: We included 86 trainees, all of female gender, 90.7% of Portuguese nationality, with an average age of 42.7 years (SD 10.6), 87.2% were formal caregivers. The average score of the questionnaire before training was 75.4%, with items from the rotation of the local administration of insulin (43.0%), foot care (55.0%) and blood glucose monitoring (57.0%), those where the students had more difficulty. The evaluation at the end of training was 95.1% and after 30 days 93.9%. There is statistical evidence that the training increased the knowledge of the trainees in the area of diabetes ($p < 0.001$).

Conclusion: We found that the knowledge of students increased significantly in the area of diabetes. The realization of these training courses may lead to an improvement of care for elderly dependents.

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The health educational juggler: exploring needs for competences in health care professionals

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Background and aims: Patient education is a crucial element in treatment and care of patients with Type 2 diabetes and other chronic illness. Because of lack of significant effect patient education needs new approaches. The literature indicates empowerment and participation are promising new approaches. However, working with participatory methods is not part of the curriculum and experience among health care professionals like nurses, dieticians, physiotherapists etc. Health care professionals need to be capable of using participatory and empowerment based methods. The need for this competence development was explored in order to design a model for competence development among health care professionals with the aim of providing participatory patient education for patients with chronic illness.

Materials and methods: Action research was used and qualitative data was collected in three workshops with 25 health care professionals (physiotherapists, nurses, occupational therapist, and dieticians) from patient educational settings in four Danish municipalities. The workshops were conducted from

November – January 2011. Data was collected through visual materials and group discussions focused on patient needs. Observation of patient education, written reflections and Story Dialogues were performed and videotapes, quotes and field notes were obtained. The analyses had a Grounded Theory approach; data was analysed by three researchers in three steps: 1) open coding, 2) thematic categorization, and 3) selective and theoretical coding including health educational theories on mediating roles, the transtheoretical model of change, comfort zones and facilitation. This led to the configuration of the main category and roles that finally generated the competence model.

Results: A competence model with four health educational roles was generated: a) the embracer (the emphatic educator), 2) the facilitator (the facilitating educator), 3) the translator (the professional educator), and 4) the initiator (the inspiring educator). These four health educational roles focus on a necessary juggling between roles which is crucial for managing genuine patient involvement that leads to empowerment. The empirical findings pointed out the embracer as the dominating strength and the facilitator, the initiator and the translator as dominating weaknesses among the health care professionals. To enhance patient education as a Health Educational Juggler health care professionals need development of competences in juggling with these roles. **Conclusion:** The study indicates that health care professionals conducting participatory patient education need to develop specific competences to be able to juggle with the professional roles in performing participatory patient education. Furthermore they need to take in new practices, reflecting on individual strengths and weaknesses as a part of the ongoing competence development. The model “The Health Educational Juggler” can be used as an analytical tool in the planning phase of participatory patient education, and as a model for competence development for health care professionals.

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A 12-month audit of the intensive weight management programme run at a large teaching hospital's diabetes centre

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Background and aims: NICE recommends health care professionals should make preventing and managing obesity a priority. We have set up a multidisciplinary intensive weight management service which includes setting weight loss goals, dietary advice, medication review and physical activity. The aim of the audit was to investigate if patients attending the program show a reduction to their weight, HbA1c and body mass index (BMI).

Materials and methods: A retrospective audit of all patients who attended the weight management program between January 2009 and July 2010 was performed comparing the data at baseline and then again at 12 months. The data was obtained from the trust diabetes database. The intensive weight management service consists of multidisciplinary consultant lead appointments where patients were enrolled into the program and their management plan agreed. This was followed by joint monthly review by DSN and Dietitian and further consultant review at 6 months. In addition patients were referred to the local Exercise for Health Program.

Results: Data was analysed using a paired t-test. Significance is $p < 0.05$. Significant improvements were seen in weight 122.45kg (SEM± 2.96) to 102.40kg (SEM±3.08) $p=0.001$ and BMI 42.81kg/m³ (SEM± 1.00) to 37.88kg/m³ (SEM±0.94) $p=0.001$. No significant changes were found in HbA1c 8.05%(SEM±0.27) to 7.59%(SEM± 0.30) $P=0.297$.

Conclusion: The audit showed that patients who attended this intensive weight management program achieved significant improvements in both weight and BMI. Although the HbA1c improved this did not achieve significance, this may possibly be explained because some patients who had their insulin discontinued when commencing incretin mimetic therapy showed a deterioration in HbA1c levels.

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Persistence of initial treatment with metformin and/or sulphonylureas in patients with type 2 diabetes

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Background and aims: Adequate persistence of oral antidiabetic treatment is highly important to achieve proper glycaemic control in patients with type 2 diabetes. The aim of the study was to evaluate the persistence of initial treatment with metformin and/or sulphonylureas in patients with type 2 diabetes. **Materials and methods:** Using the database of the Hungarian National Health Insurance Fund Administration, the persistence of initial treatment with metformin and/or sulphonylureas was evaluated between 2007–2009 in a large population ($n = 256,384$) and the results were compared to treatments with drugs widely used in cardiovascular prevention (statins, clopidogrel). Persistence was defined as the duration of time from initiation of therapy with metformin and/or sulphonylureas to discontinuation of any antidiabetic therapy. A permissible gap of 180 days based on the pharmacologic properties of the drug and the treatment situation was allowed between prefills of prescriptions. The results are reported as the rate of persistent patients at the predefined time period (12 months) in the given cohort.

Results: In patients with metformin-monotherapy ($n=115,426$) the persistence was 47.7% while the persistence was 45.4% in patients with sulphonylurea-monotherapy ($n=125,362$). In patients with metformin and sulphonylurea combination therapy ($n=15,596$) the persistence was as high as 55.8%. The treatment with modified release sulphonylureas had a higher persistence rate (47.8%) than that of traditional sulphonylureas (42.2%). The persistence of therapy using metformin 1000 mg - 60 tablets was significantly better (60.4%) at 12 months than that of other forms of metformin therapy with lower dose and smaller box analyzed together (47.7%). The persistence of therapy using metformin 1000 mg - 60 tablets was the best in patients aged between 40 and 70 years (60–66%), the persistence was reduced both in patients over 70 years of age (58%) and less than 40 years of age (20–50%). The persistence data of antidiabetic drugs proved to be higher than that of lipid lowering therapy (number of statin users 407,323, persistence: 26.3%) but lower than that of clopidogrel treatment in patients with percutaneous coronary intervention (number of clopidogrel users 20,697, persistence 73.2%).

Conclusion: Our data indicate that the persistence of initial treatment with metformin and/or sulphonylureas is far from optimal in patients with type 2 diabetes. Better diabetic care and continuous patient education (regular control, perception of treatment benefits, decrease in treatment complexity, higher use of drugs with minimal side effects, appropriate reimbursement) should be encouraged to achieve higher persistence of oral antidiabetic treatment in patients with type 2 diabetes.

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The PANORAMA European study: biomedical and psychological characteristics of people with type 2 diabetes with and without obesity

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Background and aims: PANORAMA, a large European cross-sectional study of patients (pts) with type 2 diabetes (T2D) assessed patient reported outcomes (PROs), treatment patterns and glycaemic control. This analysis compares results between obese (body mass index, BMI ≥ 30) and non-obese (BMI < 30) pts.

Materials and methods: 5817 pts ≥ 40 y, with a diagnosis of T2D for > 1 y prior to study entry and an available medical record of > 1 y, were randomly or consecutively selected from physician practices (mainly primary care) in 9 countries. All pts received lifestyle advice. Most pts were also being treated

with either oral antidiabetes agents (OADs) or injectables (insulin or GLP-1 analogues) with or without OADs. Treatment type was unchanged in the previous 3 months. Pts completed the Audit of Diabetes-Dependent Quality of Life (ADDQoL), Diabetes Treatment Satisfaction Questionnaire (DTSQ), worry subscale of the Hypoglycemic Fear Survey-II (HFS-II) and EuroQoL-5 Dimension (EQ-5D) index and visual analogue scale.

Results: 45.6% of pts were obese. Obese pts were younger (64.2 ± 10.1 y vs 67.3 ± 10.4 y; $p < 0.001$) and characterised by shorter diabetes duration (8.5 ± 6.8 y vs 9.2 ± 7.4 y; $p < 0.001$). The obese group included more women (52.4% vs 41.2%; $p < 0.001$), fewer current smokers (12.8% vs 15.6%; $p = 0.002$) and were less educated (education after 18 y: 27.4% vs 32.3%; $p < 0.001$). Glycaemic control was worse in obese pts as shown by the proportion with $HbA_{1c} < 7\%$ (57.1% vs 67.3%; $p < 0.001$) and mean HbA_{1c} ($7.0 \pm 1.2\%$ vs $6.8 \pm 1.1\%$; $p < 0.001$). Treatment regimens were more intensive for obese vs non-obese pts: lifestyle advice alone (8.5% vs 11.1%, $p = 0.001$), 1 OAD (31.0% vs 33.2%; $p = 0.067$), 2 OADs (26.2% vs 27.6%; $p = 0.23$), 3 OADs (9.9% vs 8.1%; $p = 0.019$) and insulin \pm OAD (22.1% vs 18.5%; $p = 0.001$). Physicians rated pts' adherence to treatment and lifestyle advice as good in 66.0% vs 73.5% and 26.2% vs 49.5% of obese vs non-obese pts (both $p < 0.001$). Obese pts had higher systolic (136.1 ± 15.5 vs 133.3 ± 15.1 mmHg) and diastolic (79.5 ± 9.5 vs 77.3 ± 8.8 mmHg) blood pressure than non-obese pts (both $p < 0.001$). Obese vs non-obese pts had a greater prevalence of depressive disorders (16.0% vs 11.9%; $p < 0.001$), sleep disorders (17.5% vs 11.7%; $p < 0.001$) and microvascular complications (30.5% vs 26.7%; $p = 0.001$). Obese pts showed more negative impact of diabetes on QoL (ADDQoL), less treatment satisfaction (DTSQ), greater fear of hypoglycaemia (HFS-ws) and worse health status (EQ-5D) than non-obese pts (all $p < 0.001$).

Conclusion: Obesity in pts with T2D was associated with a greater prevalence of co-morbidities and microvascular complications, and poorer glycaemic control. Treatment regimens differed somewhat between obese and non-obese pts. Physicians rated obese pts as less likely to adhere to lifestyle advice and treatment. PRO measures showed more negative responses in obese pts. These results highlight the importance of effective weight management in the treatment of pts with T2D, and suggest that improved efforts to tailor treatments to individual lifestyles may be beneficial for obese pts.

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PS 081 Psychological aspects: type 1 diabetes

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Parental fear of hypoglycaemia in mothers of children with type 1 diabetes mellitus: a cultural comparison

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Background and aims: Fear of hypoglycemia (FoH) can affect quality of life and diabetes management and is prevalent, not only in patients with type 1 diabetes (T1D), but also in parents of children with T1D, especially mothers. While maternal FoH has been studied in several different countries and appears to have global relevance, no studies have yet investigated cultural similarities and differences. This study compared FoH in mothers of T1D children in the U.S., Norway and Iran.

Material and methods: FoH was measured with the parent version of the Hypoglycemia Fear Survey (PHFS), composed of a behavior (PHFS-B) and worry (PHFS-W) subscale, measuring, respectively, behaviors to avoid and worries about hypoglycemia or its negative consequences. The PHFS was completed by mothers of children with T1D ranging in age from 2-18 years in separate studies conducted in the U.S. ($n = 207$), Norway ($n = 103$) and Iran ($n = 60$). All parents also completed a demographic/clinical questionnaire, and their child's HbA_{1c} was measured.

Results: There were no differences in PHFS-W scores across countries, but Iranian mothers had higher mean item scores ($F = 6.5$, $p = .002$) on the PHFS-B ($\mu = 2.7$) compared to U.S. and Norwegian mothers ($\mu = 2.4$ and 2.3). The percent of mothers with PHFS item scores more than 1 SD higher than overall mean score did not differ between countries for either subscale (range for both 12.2-18%). PHFS scores did not differ for mothers of girls vs. boys for any country. Bivariate analysis found no relationship between PHFS scores and HbA_{1c} for any country, although Iranian children had significantly higher values than U.S. and Norwegian children ($F = 37.8$, $p = .000$). Mothers who reported more frequent hypoglycemia in their child scored higher on the PHFS-B for the U.S. ($p = .000$), with a trend for Iran ($p = .051$), and on the PHFS-W for Norway ($p = .025$). Exploratory factor analysis (EFA) showed the best fit for a four-factor solution for both the U.S. ($\chi^2_{(70)} = 136.2$ ($p < .00005$), RMSEA = 0.07, SRMR = 0.05, CFI = .95, TLI = .97) and Norway ($\chi^2_{(53)} = 84.3$ ($p < .00005$), RMSEA = 0.08, SRMR = 0.07, CFI = .94, TLI = .96). There was insufficient Iranian data for EFA. These four factors were generally similar for U.S. and Norwegian data, with two PHFS-B factors describing behaviors to 1) keep the child's blood glucose levels higher in some situations and 2) prevent hypoglycemia. The two PHFS-W factors described worries about the 1) child not having help or food available and 2) social and other negative consequences of hypoglycemia.

Conclusions: Mothers of children with T1DM in the U.S., Norway and Iran showed equivalent levels of worry about hypoglycemia and, in each country, mothers with female and male children worried equally. Iranian mothers, however, reported more behaviors to prevent hypoglycemia and its negative consequences. Preliminary analysis found no relationship between maternal FoH and HbA_{1c} levels, but FoH was related to children's frequency of hypoglycemia. Factor analysis results suggest that FoH is a complex construct and, as measured by the PHFS, comprised of four separate subtypes of behavioral and affective responses to hypoglycemia which are relatively consistent across the U.S. and Norway. Maternal FoH appears to have global relevance and similarities across different cultures.

Clinical Trial Registration Number: NIH/NIDDK

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Diabetes and teenagers: psychological aspects

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Background and aims: Diabetes is one of the most common chronic diseases whose incidence is increasing steadily, it is categorized as a “social pathology”. When diabetes occurs in adolescent age, the patient must address not only the biological changes, psychological and social factors that characterize this developmental stage, but also the “challenges” that arise daily in the management of the disease. The aim of this study was to identify the specific areas of social life most affected by type 1 diabetes mellitus, in order to facilitate the implementation of psychological interventions aimed to reduce the state of discomfort experienced by patients.

Materials and methods: We enrolled 48 subjects (21 males and 27 females) with an age between 12 and 17 years, all affected by type 1 diabetes mellitus. We administered the PAID-T (Promemetic Areas in Diabetes Scale-Teen) questionnaire to evaluate the psychological implications of diabetes mellitus between teenagers. The questionnaire was composed by 26 items clustered in 4 subgroups: “Emotional tension” (TE), indicates the feelings of discomfort and emotional reactions related to the chronicity of diabetes and the perceived limits; “Self-efficacy” (AE), refers to the perception of “being able to manage the disease”; “Interpersonal discomfort” (DI), indicates the absence of social support, with particular reference to the quality of support provided by family and friends; “Discomfort for diet” (DA), concerning issues related to diet. Subjects were asked to indicate the degree of distress or discomfort created by any potential situation contained in the 26 items in the last month by circling the appropriate number on a Likert scale with 6 levels.

Results: Comparing the answers regarding the four groups of questions, we did not observe any statistically significant differences between males and females. Both males and females referred to experience a greater distress related to diet therapy, probably because the food is perceived as a “constraint” to which young people rebel against. In this subscale followed, with a slightly lower score, the existential distress related to being affected by this chronic disease which affects daily choices and actions. From the analysis of the scores obtained in the 4 subscales, emerged that adolescents show a similar level of discomfort for being people with diabetes and for the need of having to restrict the range of foods to eat, which generates the consequential discomfort for the compliance to a balanced diet. There was a significant correlation between emotional reactions (TE) and sense of self-efficacy experienced by subjects (AE), and a positive correlation between the last and the absence of socio-familial support (DI) and discomfort due to the need to undergo a nutritional therapy (DA).

Conclusion: The study is considered a useful systematic psychological consultation for patients with diabetes and, especially, for teenagers, in order to plan therapeutic interventions targeted at specific problematic areas that trouble them and produce severe consequences in the relationship with the physician team and the families.

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A comparison of quality of life measured with the SF-36 for insulin degludec and insulin glargine in people with type 1 diabetes

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Background and aims: Insulin degludec (IDeg), a new-generation basal insulin, forms soluble multi-hexamers upon subcutaneous injection resulting in an ultra-long action profile. The efficacy and safety of IDeg in people with type 1 diabetes have been investigated in comparison with insulin glargine (IGlar) in a 16-week, open-label, randomised trial. Quality of life is an important aspect of efficacy in people with diabetes.

Materials and methods: Efficacy assessment utilised the validated SF-36 v.2 questionnaire, which has a two summary component scores for mental (MCS) and physical (PCS) well-being, each of four domains. IDeg (n=59) or IGlar (n=59) were injected once daily in the evening, with insulin aspart at mealtimes. At baseline, participants had a mean age of 45.8 yr, mean HbA_{1c} of 8.4 % (68 mmol/mol) and mean BMI of 26.9 kg/m², while after 16 weeks HbA_{1c} was comparable (IDeg-IGlar difference 0.10 [95% CI -0.14; 0.34] % units [1.1 [-1.5; 3.7] mmol/mol), confirmed nocturnal hypoglycaemia was less with IDeg (relative rate (RR) 0.42 [0.25; 0.69]), and overall hypoglycaemia (plasma glucose <3.1 mmol/l or requiring assistance) numerically less frequent (RR 0.72 [0.52; 1.00]).

Results: After 16 weeks, a significant improvement in SF-36 MCS of +3.01 [0.32; 5.70] was obtained for IDeg against IGlar. The difference in MCS was driven by significant differences in the social functioning (SF) domain (+8.04 [1.89; 14.18]) and the mental health (MH) domain (+2.46 [0.10; 4.82]). For MCS, Cohen's effect size was 0.42, indicating a small to medium clinically meaningful difference. SF-36 PCS was similar between insulins (+0.66 [-2.30; 3.62]). The remaining six SF-36 sub-domains showed no significant differences between IDeg and IGlar.

Conclusion: We hypothesise that the improvements in overall MCS and the underlying SF and MH domains with degludec compared to glargine relates to the observed reduction in hypoglycaemic events, but greater flexibility in the time of the basal insulin injection might also contribute. In summary, in the context of comparable overall glycaemic control to IGlar, IDeg gave improved mental well-being as measured using the MCS component of the SF-36 questionnaire.

Clinical Trial Registration Number: NCT00612040

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Insulin manipulation as an indicator of psychiatric co-morbidity in children and adolescents with type 1 diabetes mellitus

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Background and aims: Deterioration of metabolic control is a frequent occurrence in adolescent diabetics. Reasons cannot only be explained by physiological changes in puberty. Psychological factors contribute to this deterioration. Psychiatric co morbidity is known to be connected to worsening of metabolic control although the mechanisms are not completely clear. Non-compliance with diabetes therapy is regarded as an important factor. Insulin purging is widely known as a symptom in eating disorders in diabetes, while in contrast deliberate overdosing of insulin seems to be less frequent. In our study we aimed to assess the rate of insulin manipulation and a possible association with psychiatric co morbidity and metabolic control.

Materials and methods: 241 type 1 diabetes patients (age range 10-22 years) with a diabetes duration of >1 year from 21 diabetes outpatient clinics in Austria were included in the cross sectional study. 103 (42.7%) of the patients were males. Medical data was derived from medical records. Patients were interviewed with standardized instruments, the Diabetes Self-Management Profile (DSMP) interview and the Kinder-DIPS. T-Test, U-Test and CHI2-test were used for comparison.

Results: Based on the DSMP-Interview adherence to prescribed insulin dosage was assessed. 42.7% of the patients (n=103) were found to be compliant, 27.8% (n=67) had management problems (unintended over- or under-dosage of insulin) and 29.5% (n=71) patients reported deliberate insulin manipulation in terms of under-dosing (24.1%) and over-dosing (22.8%) their insulin. With the Kinder-DIPS psychiatric co morbidity was screened in all patients. Patients with management problems and compliant patients did not show any elevation in prevalence of psychiatric co morbidity. In the manipulating subjects we found elevated levels of depression (5.6%vs 1.0%, p<.05), specific phobia (7.0%vs 1.0%,p<.05), social phobia (5.6%vs 0%,p<.05) and eating disorders (EDNOS 7.0%vs 0%, p<.05) and highly elevated subclinical specific phobia (11.3%vs 0%, p=.001). HbA1c levels were higher among the manipulating patients (8.65%vs 7.81%, p=.001), their BMI was elevated but not significantly (BMI-SDS .176vs -.191, p=.06). Manipulating patients had more often ketoacidosis with the need of hospitalization in the past year (34.2%vs 14.7%, p<.05). Manipulating subjects were significantly older (15.00 years) than compliant subjects (14.00 years, p=.004) and more girls (69%) than boys (31%) were found in the manipulating group (p=.001).

Conclusion: Insulin manipulation can be surmised as a psychological parameter and as an indicator of psychiatric co morbidity in adolescents with diabetes. Older girls are more at risk and there is a significant impact on metabolic control. Further research is needed, especially a screening tool for insulin manipulation.

Clinical Trial Registration Number: 12673

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Differences in perceptions of diabetes responsibility and dietary self-efficacy in relation to adolescent and parental diabetes distress

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Background and aims: Having to care for diabetes is often stressful to both adolescents with type 1 diabetes (T1DM) and their parents. Negative beliefs about diabetes and its perceived consequences, lack of self-efficacy, conflicts regarding responsibility for self-care activities, and poor diabetes control may all contribute. However, few studies have examined the role of differences in perceptions between adolescents and their parents regarding diabetes responsibility and self-efficacy in relation to distress. The aim of this study was to examine whether differences in perception of dietary self-efficacy and diabetes responsibility are associated with adolescent and parental diabetes distress.

Materials and methods: 213 adolescents with T1DM (47% male) and one of their parents, completed questionnaires assessing dietary self-efficacy, illness beliefs, diabetes distress, self-care activities and family responsibility. Demographics and illness-related variables, BMI and HbA1c were also assessed.

Results: Hierarchical regression showed that when controlling for age, sex, HbA1c and duration of diabetes, diabetes distress of adolescents with T1DM was significantly predicted by their dietary self-efficacy beliefs ($\beta = -0.38$, $p < 0.0001$) and beliefs that diabetes has negative consequences ($\beta = 0.19$, $p < 0.01$). Disagreements regarding diabetes self-care and self-efficacy between parent and adolescent were not significant predictors of adolescent distress (all $p > 0.30$). The model explained 23% of the variance in adolescent diabetes distress. Parental diabetes distress was significantly predicted by parent perception of adolescent dietary self-efficacy ($\beta = -0.42$, $p < 0.0001$), and by diabetes responsibility disagreements when both claim responsibility ($\beta = 0.15$, $p < 0.04$). Neither differences in dietary self-efficacy nor diabetes responsibility when no one claimed responsibility were significant predictors of parental distress ($p > 0.40$). The model explained 25% of the variance in parental diabetes distress.

Conclusion: For adolescents with T1DM, diabetes distress was associated with a lack of confidence in their ability to follow the diet and a belief that doing so would lead to negative consequences. Disagreement between parent and adolescent with regard to self-efficacy and self-care is not related to distress in the adolescent. On the other hand, when parents doubt the ability of their adolescent to manage the diet and/or when there is disagreement regarding self-care activities, such that both claim responsibility, parents are more distressed. Parental distress is therefore likely to result from conflict surrounding these disagreements. When there is disagreement with no one claiming responsibility, parental distress is not increased possibly because it does not lead to conflict.

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Psychological interventions in a specialised diabetes hospital: differences between patients with type 1 and type 2 diabetes

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Background and aims: Psychological problems are common in patients with type 1- and type 2-diabetes. Especially during in-patient diabetes treatment, a psychological intervention is often a mandatory component of therapy. The aim of this study is to evaluate possible differences in psychosomatic diagnoses applicable to patients with type 1- and type 2- diabetes and which problem areas connected with them.

Materials and methods: Over 14 months, 161 out of 848 treated patients with type 1-diabetes (19.0 %) and 252 out of 1725 patients with type 2-diabetes (14.6 %, $p = 0.0053$) received psychological evaluation and treatment. Patients with type 1- and type 2-diabetes differed regarding age (42 ± 15 vs. 58

± 12 yrs., $p < 0.0001$), BMI (26.1 ± 7.6 vs. 34.8 ± 8.3 kg/m², $p < 0.0001$), but not concerning gender distribution (m/f: 75/86 vs. 118/134, $p = 1.0$) and HbA_{1c} (9.5 ± 2.1 vs. 9.1 ± 1.8 %, $p = 0.10$). Statistical analyzes: χ^2 -test, ANOVA, results were reported as proportions (%), mean values and standard deviations.

Results: The most common diagnoses in both groups were neurotic, stress-related and somatoform disorders (ICD F 4), followed by mood (affective) disorders (ICD F3; slightly more common in type 2-diabetes; $p = 0.24$), behavioral syndromes associated with physical factors (ICD F5; more common in type 1-diabetes, $p = 0.022$) and disorders due to psychoactive substance use (ICD F1; more common in type 1-diabetes, $p = 0.016$). Regarding age distribution the patients with type 1- and type 2-diabetes differed significantly ($p < 0.0001$), but the distribution of diagnoses among particular age groups showed no significant differences. Commonly articulated problem areas were family conflicts (25.4 %), coping with the illness (24.0 %), job problems (14.3 %) and dealing with grief (8.7 %). In 25.9 % (type 1-diabetes: 30.4 %, type 2-diabetes: 23.0 %; $p = 0.11$) a further psychotherapeutic out-patient treatment and in 1.2 % an in-patient treatment was recommended.

Conclusion: Psychiatric disorders are common in patients at a specialised diabetes hospital and often need psychological, psychosomatic and psychotherapeutic treatment during the in-patient treatment and afterwards. The distribution of the psychiatric diagnoses is similar in patients with type 1- and type 2-diabetes, especially when regarding the age groups. Similarly, there is no obvious difference in problem fields between patients with type 1- and type 2-diabetes, especially when age categories are taken into account.

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Non-healthy food of higher psychosocial value than healthy nutrition? Insights from a European study on overweight children and teenagers

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Background and aims: Obesity is a growing health epidemic in developed and developing countries and consequently the risk of developing diabetes at early ages. New tendencies concentrate on the prevention of overweight and therefore focus on children and teenagers. Consequently the right strategy in the treatment and education of overweight children and teenagers and their parents becomes increasingly important. Healthy nutrition is one of the basic formats therapy is based on. The question is whether we have to redefine what is understood as healthy food, in terms of considering not only the nutritional values, but also the psychological and social value of food.

Materials and methods: We undertook a qualitative research in 5 European countries (Germany, UK, France, Italy, Spain) to obtain insight into attitude and expectations of mothers of overweight children and teenagers (N=90), children and teenagers (N=81) and the involved physicians (N=20) and health care authorities (N=15).

Results: Our study revealed changing eating habits among children and teenagers is far more complex than just becoming accustomed to eating more balanced meals. Apart from a psychological component (good taste, joy, self esteem) a strong psycho-social dimension was detected. Food is a link between mothers and children with daily meals and snacks used to maintain this social connection. By offering the children's preferred food mothers establish an ongoing interchange of reward, understanding and affection. Motivational barriers to change nutritional habits of mothers and children/teenagers are strongly influenced by this psycho-social dimension. Physicians emphasized the importance to increase the success rate of the therapy but at the same time mentioned a lack of resources to be able to consider the complex image of nutrition in all its dimensions. Present strategies are often based on reducing eating habits without offering a substitution for the missing psychosocial values to families. Thus, successful new strategies should focus on adding immediate psychosocial values to the children's and teenager's life (e.g. family events including physical activity) that compensate perceived negative changes (food reduction).

Conclusion: To successfully address the challenge of overweight among children and teenagers, and to reduce the risk of diabetes among children, teenagers and adults, we need to consider the psycho-social dimension of nutrition and find ways to substitute the role certain food plays in this sense, with positive experiences relating to an active family life.

Supported by: Omron Healthcare Europe

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The effect of deprivation and HbA_{1c} on hyperglycaemic admission to hospital in type 1 diabetes

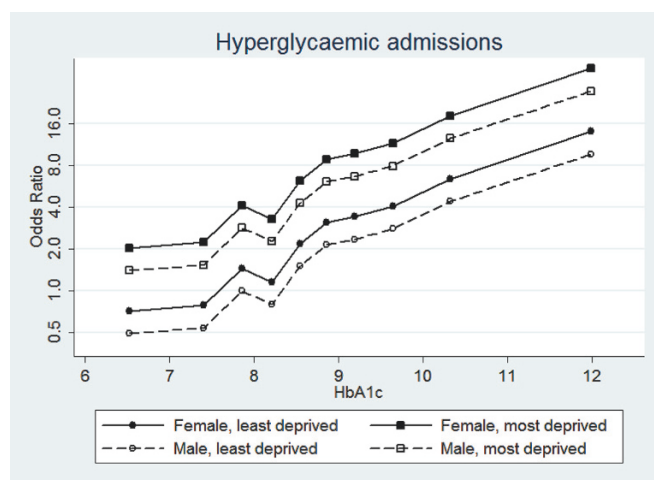
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Background and aims: HbA_{1c} may be a useful tool in defining that part of the population likely to suffer morbidity and therefore incur an increase in hospital admissions in the short and medium term. We have previously found a positive association between increasing HbA_{1c} levels and risk of hospital admission in the Scottish type 1 diabetes population. In the general population deprivation has also been linked to increased risk of admission to hospital. This study aims to examine how deprivation affects admission to hospital for people with type 1 diabetes in Scotland.

Materials and methods: The Scottish Care Information - Diabetes Collaboration (SCI-DC) is a dynamic national register of diagnosed cases of diabetes in Scotland. These data were linked to centralised data on hospital admissions from Information Services Division (ISD) of NHS National Services. We identified 24,760 people with type 1 diabetes during January 2005 to December 2007 and include 21,436 patients with complete recording of covariates. Patients were divided into deciles according to levels of HbA_{1c}. Hyperglycaemic admission to hospital was the primary outcome (diabetes with ketoacidosis, ICD10: E10.1, E14.1). Logistic regression models were used to estimate the association between hyperglycaemic admissions and HbA_{1c} (expressed with decile 3 as referent, mean HbA_{1c} 7.86%, range 7.66%–8.05%), deprivation quintiles (referent quintile 1, least deprived), and adjusted for potential confounding factors including smoking status, age, sex, previous vascular disease, creatinine, body mass index and diabetes duration.

Results: There was a J-shaped relationship of HbA_{1c} to hyperglycaemic admissions (Figure) with highest likelihood of admission in the highest HbA_{1c} decile (adjusted odds ratio 9.68, 95% confidence interval 7.13–13.13). People in the top decile of HbA_{1c} (10.8%–18.4%) were younger (average 24 (sd 11) for females and 27 (sd 12) for males vs 38 (sd 10) and 45 (17) in decile 3). Even with adjustment for other covariates including HbA_{1c}, females had a consistently higher risk of admission compared to males (OR 1.45, 95%CI 1.28–1.65). There was a substantial effect of socioeconomic class on odds of admission. Compared to the least deprived quintile, odds of admission increased with deprivation (OR 2.84, 95%CI 2.32–3.47 for most deprived). Compared to the least deprived quintile, the most deprived quintile had, on average, an additional 194 hyperglycaemic admissions per year in the top HbA_{1c} decile (>12%). Assuming an average cost of £1702 per hyperglycaemic admission, deprivation accounts for an additional £330k per year.

Conclusion: While for all groups, high mean HbA_{1c} values predict admission to hospital for hyperglycaemia, females and people from more deprived parts of the community have a further increase in admissions. This work highlights a group who might be usefully supported to try to reduce hospital admissions.



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Cerebral resting-state default mode network changes in patients with type 1 diabetes with and without microangiopathy relate to cognitive functions

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Background and aims: At rest, the brain shows consistently active regions forming circumscribed neuronal networks detectable using resting-state functional magnetic resonance imaging (RS-fMRI). One such network, the Default Mode Network (DMN), is considered to be especially important for cognitive functions. This network consists of the medial prefrontal cortex, posterior and anterior cingulate and precuneus, superior parietal cortex, and temporal regions such as the hippocampus. Activity, or synchronisation, in the DMN in for example Alzheimer's disease and multiple sclerosis, increases in the early phases of the disease and decreases with disease progression. As type 1 diabetes (T1DM), in particular in the presence of microangiopathy, is associated with cognitive decline, DMN synchronisation changes might be expected, but this has hitherto not been investigated. We assessed DMN activity in T1DM patients with and without microangiopathy and matched controls and the correlation between DMN synchronisation and cognitive functions.

Materials and methods: Forty-nine T1DM patients with, 52 T1DM patients without microangiopathy and 48 controls underwent cognitive testing for general cognitive ability, memory, information processing speed, executive functions, attention, motor- and psychomotor speed, and MRI, including a 3D T1 structural and a RS-fMRI scan. The DMN was identified using MELODIC, part of FSL 4.1 software. Group differences were assessed on a voxel level using dual regression. Analyses were corrected for age, gender, depressive symptoms and multiple comparisons. Mean DMN synchronisation in patients was used to determine its relationship with cognitive functions and disease related variables.

Results: T1DM patients with microangiopathy were significantly older, had higher HbA_{1c} levels, depression scores and performed worse on cognitive testing, as compared to the other groups (all $P < 0.05$). Across all subjects, DMN synchronisation was found in the areas described above. In patients, synchronisation of DMN was positively associated with information processing speed ($r = 0.266$; $P = 0.023$) and negatively with age and T1DM onset age ($r = -0.297$; $P = 0.002$ and $r = -0.202$; $P = 0.042$). When comparing all groups on DMN synchronisation, an increase was found in the right middle temporal gyrus in T1DM without microangiopathy relative to controls ($P < 0.05$), but not in T1DM with microangiopathy.

Conclusion: We found DMN synchronisation indeed correlated with cognitive functions and that on group level this synchronisation is slightly different in T1DM patients without microangiopathy compared to controls. These results seem in line with the compensatory mechanism to prevent cognitive decline seen in other neurological diseases. Further study needs to determine possible changes in DMN synchronisation over time and its exact impact on cognition.

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The association between adherence to insulin prescriptions and HbA_{1c} among patients with type 1 diabetes

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Background and aims: The degree of adherence to the prescribed insulin regimen is thought to play an important role in determining long term glycaemic regulation among patients with type 1 diabetes (T1DM). Our aim was to quantify the effect of adherence to insulin prescriptions on HbA_{1c} based on individual record linkage of electronic records.

Materials and methods: We studied 3,807 T1DM patients followed up between 1998 and 2009, some patients with electronic recordings of having been referred as early as 1996. Measurements of HbA_{1c} and registrations of prescribed daily doses at the T1DM outpatient clinic in a specialised diabetes hospital since 1998 were linked to records of filled prescriptions from the Register of Medicinal Product Statistics supplied from the Danish Medicines

Agency. The degree of insulin adherence was calculated as the proportion of days a patient had possession of sufficient insulin to cover his or her prescription. Adherence as well as the average prescribed daily dose of insulin during the 120 days prior to each HbA_{1c} measurement was considered comprising a total of 39,526 observations, with each patient contributing multiple HbA_{1c} measurements. A standard linear random coefficient model was used for analysis.

Results: At the end of 2009 the 3,807 patients (54.3 % men) had had a median attendance at the hospital of 12.3 years (minimum 0.1 and maximum 14.0 years). Mean age at beginning of follow-up in 1998 was 40.8 years (SD: 16.7 years) with a mean duration of diabetes of 16.7 years (SD: 13.3 years) at referral to the outpatient clinic. A 0.1 (10%) higher degree of adherence to the prescribed insulin dose was associated with a 0.006 percentage point (95% CI: -0.011 ; - 0.001) lower HbA_{1c}. However, the variation between patients of the estimate showed a SD of 0.254, indicating non-homogeneity. Eighty three % of the patients had a degree of adherence to insulin prescriptions of 1.0 and the 10th percentile was 0.39. There was a significant negative association between average prescribed daily doses and HbA_{1c}, in accordance with prescription of increased doses to patients with poor glycaemic control.

Conclusion: As the level of HbA_{1c} and the prescribed dose are interdependent in a continuous, iterative process consisting of an HbA_{1c} measurement and subsequent adjustment of the prescribed dose, a traditional approach to estimation of associations between medication adherence and glycaemic control is problematic. Methods based on changes in prescribed dose and adherence will need to be developed.

PS 082 Psychological aspects: type 2 diabetes

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Change in health status (EQ-5D) over 5 years among individuals with and without diabetes

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Background and aims: This study assessed the change in health status and health-related quality of life over 5 years among individuals with and without diabetes.

Materials and methods: Respondents to the US Study to Help Improve Early evaluation and management of risk factors Leading to Diabetes (SHIELD) surveys completed the EuroQol-5D (EQ-5D) quality-of-life questionnaire at baseline (2004) and 5 years later (2009). The two EQ-5D components (visual analog scale [VAS] score and the health index score) were computed at baseline and year 5, and the change over 5 years was measured for individuals with type 2 diabetes (T2DM) and those without diabetes. Linear regression models were used to determine change in EQ-5D score controlling for age, gender, race, education, household income, body mass index (BMI), and diabetes status (T2DM, no diabetes).

Results: For respondents with T2DM (n = 1,741), 60% were women and mean age was 61 years compared with 62% women and mean age of 56 years for respondents without diabetes (n = 4,543). There was a significantly greater decline in the EQ-5D index score in the T2DM group (-0.031 [SD 0.158]), compared with those without diabetes (-0.016 [0.141]) over the 5-year period (p = 0.001). Compared with respondents without diabetes, those with T2DM had a larger reduction in EQ-5D index score, after controlling for age, gender, race, education, income, and BMI (p = 0.001). The EQ-5D VAS score declined over 5 years for both groups: -1.42 (18.1) for T2DM group, and -0.63 (15.8) for group without diabetes, but the difference was not significant either before (p = 0.09) or after (p = 0.12) controlling for demographics (p = 0.12). Among the T2DM respondents, the decline in EQ-5D index score was significantly greater among those who had reported diabetes complications over the 5 years, compared with those without complications: -0.058 for retinopathy vs. -0.028 for no retinopathy (p = 0.02), and -0.061 for neuropathy vs. -0.020 for no neuropathy (p < 0.001).

Conclusion: Over a 5-year period, health status of respondents with T2DM declined significantly, compared with those with no diabetes, indicating that the burden of the disease has a long-term detrimental impact on the quality of life of patients living with type 2 diabetes.

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Validation of the (short) Exercise Self-efficacy Scale (ESS) in Dutch primary care patients with type 2 diabetes mellitus

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Background and aims: Exercise is an essential element of type 2 diabetes (T2DM) management, as it can lower the risk of complications. However, an estimated 60-70% of T2DM do not exercise enough. One of the psychosocial constructs that is believed to influence physical activity behaviour, is exercise self-efficacy. Exercise self-efficacy is the extent to which people believe they can maintain or improve their level of physical activity. The purpose of this study is to validate the Dutch (short) version of the Exercise Self-efficacy Scale (ESS) in a T2DM Primary Care sample.

Materials and methods: T2DM patients (n=322) with a BMI >25 and <80 years old filled out the ESS and the Short Questionnaire to ASsess Health enhancing physical activity (SQUASH). The ESS contains 18 items describing situations that might interfere with adherence to exercise routine. The ESS rates the degree of confidence on a 100-point scale, higher scores indicating more self-efficacy. The total score of the ESS is calculated as the mean of the items. ESS construct-validity was assessed (principal axis factor analysis, Cronbach's α , inter-item- and item-total correlations). Moreover, the concurrent validity with the SQUASH outcomes 'total-' and 'leisure time minutes/week of moderate to vigorous intensity physical activity' was evaluated. As

these outcomes are not normally distributed, non-parametric tests were used (Spearman correlation and Mann-Whitney U). Finally, similar analyses were performed using a short (5-items) version of the ESS.

Results: Median total score on the ESS was 41.8 with a range between 0 and 99. A low score on the ESS was defined as a score ≤ 40 . All ESS item scores ranged from 0 to 100, without any floor- or ceiling effect. The principal axis factor analysis suggested one underlying factor, explaining 54% of the total variance. Factor loadings varied between 0.48 and 0.83. Inter-item correlations varied between 0.24 and 0.79, item-total correlations varied between 0.47 and 0.81. Cronbach's α coefficient was 0.95. The median (range) of the SQUASH outcomes 'total-' and 'leisure time physical activity' were 420 (0–4620) and 270 (0–2640) respectively. The correlations of the ESS with 'total-' and 'leisure time physical activity' were 0.18 ($p = 0.002$; effect size: 0.37) and 0.23 ($p < 0.001$; effect size: 0.47) respectively. Patients with a low ESS score (≤ 40) had lower 'total-' ($p = 0.017$) and 'leisure time physical activity' ($p = 0.003$) compared to subjects with a high ESS score (> 40). Subsequently, a short version of the ESS was developed including 5 items. Construct analysis revealed a one factor solution, explaining 69% of the variance. Factor loadings varied between 0.78 and 0.86. The item-total correlation varied between 0.75 to 0.81 with a Cronbach's α coefficient of 0.92. The short-ESS correlated strongly with the total-ESS (0.94). The correlations of the short-ESS with the SQUASH outcomes 'total-' and 'leisure time physical activity' were 0.15 ($p = 0.01$; effect size: 0.30) and 0.20 ($p < 0.001$; effect size: 0.41) respectively. Patients with a low short-ESS score (≤ 40) had lower 'total-' ($p = 0.017$) and 'leisure time physical activity' ($p < 0.001$) compared to subjects with a high score (> 40).

Conclusion: This study is the first to show the validity of the ESS in a large sample of T2DM patients. The ESS and its short version might be useful in (intervention) research on physical activity in T2DM patients.

Clinical Trial Registration Number: 2734

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Application of the STCD₂ treatment satisfaction questionnaire in a cohort of caregivers of dependent type 2 diabetic, hospitalised patients

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Background and aims: The STCD₂ questionnaire has been recently validated to ascertain the satisfaction with the treatment of the caregivers of dependent type 2 diabetic patients. Our survey was designed to analyze the data after applying this methodology in a cohort of subjects studied in a hospital setting.

Materials and methods: The eight-item STCD₂ questionnaire was applied to the caregivers of consecutive, dependent, type 2 diabetic patients receiving anti-diabetic medication, admitted (for any reason) to the internal medicine service of our hospital. The questionnaire, referred to the satisfaction with the treatment received before admission, was administered in an interview by a member of the research team during the hospitalization period. Epidemiological and clinical data of the patients, as well as epidemiological information of their caregivers was also collected and analyzed.

Results: The STCD₂ questionnaire was administered to 132 subjects. The patients they were caring for (women: $n = 87$, 65.9%; mean age \pm SD: 84.2 ± 6.8 years) came from nursing homes in $n = 48$, 36.4% cases, and had scores in the Lawton and Brody scale of mean \pm SD: 1.0 ± 1.4 points (median: 0.0 points) and in the Barthel scale of mean \pm SD: 38.5 ± 27.7 points (median: 42.5 points). At admission, they received insulin or insulin analogues in $n = 53$, 42% cases; oral anti-diabetic agents in $n = 63$, 47.7% cases; and both in $n = 16$, 12.1% cases. The number of daily administrations of anti-diabetic medication was one in $n = 59$, 44.7% cases, and ≥ 3 in $n = 36$, 27.3% cases. Mean \pm SD values of HbA_{1c} at admission were 7.3 ± 1.5 %. Surveyed caregivers (women: $n = 104$, 78.8%; mean age \pm SD: 50.5 ± 15.6 years) had a secondary or high educational level in $n = 72$, 54.6% cases, they were remunerated employees in $n = 48$, 36.4% cases, and they utilized a mean \pm SD of 12.6 ± 7.3 hours per day in the care of the dependent diabetics. They revealed to be 'very' or 'fairly' satisfied with the treatment that received the patients they cared for in $n = 92$, 69.7% cases. Regarding specific areas, the same high levels ('Very satisfied' / 'Fairly satisfied') of satisfaction were reached in: satisfaction with the 'blood sugar levels', $n = 83$, 62.9%; satisfaction with the acceptance of the treatment by the patient, $n = 94$, 71.3%; satisfaction with the easiness of administration of medication, $n = 94$, 71.3%; satisfaction with the number of administrations per day of the medication, $n = 95$, 71.9%; and satisfaction with their own knowledge about the treatment of diabetes, $n = 52$, 39.3%. Statistically significant correlations were detected between the score of global treatment satisfaction and number of administrations per day of the medica-

tion ($r = -0.27$, $P = 0.002$), and level of HbA_{1c} ($r = -0.32$, $P = 0.000$). Groups of caregivers of patients receiving short acting insulin or analogues, and insulin plus oral anti-diabetic agents showed significantly lower levels of satisfaction (χ^2 , $P = 0.000$ and $P = 0.001$, respectively). In a logistic regression analysis, HbA_{1c} numbers were identified as predictors of caregiver satisfaction (OR: 0.560, IC 95%: 0.416–0.753, $P = 0.000$).

Conclusion: Our data suggest that simplicity in anti-diabetic therapy has to be considered when addressing the management of dependent type 2 diabetic patients if satisfaction of their caregivers is an additional objective.

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Development and validation of a treatment satisfaction questionnaire for caregivers of dependent type 2 diabetic patients (STCD₂)

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Background and aims: This study was designed to develop a diabetes-specific questionnaire for caregivers to assess their satisfaction with the treatment that receive the dependent type 2 diabetic patients under their care and to conduct its psychometric validation.

Materials and methods: After a review of the literature and field tests, a questionnaire of 'Satisfacción con el Tratamiento de los Cuidadores de pacientes Diabéticos Dependientes' (STCD₂) was elaborated. The STCD₂ questionnaire was applied to the caregivers of consecutive, dependent, type 2 diabetic patients receiving anti-diabetic medication, admitted (for any reason) to the internal medicine service of our hospital. The questionnaire, referred to the satisfaction with the treatment received before admission, was administered in an interview by a member of the research team during the hospitalization period. The questionnaire was administered again, four weeks later, in a telephonic interview to estimate the instrument test-retest reliability.

Results: The STCD₂ questionnaire contains eight items directed to assess total diabetes treatment satisfaction and treatment satisfaction in specific areas (satisfaction with the 'blood sugar levels', satisfaction with the acceptance of the treatment by the patient, satisfaction with the easiness of administration of medication, satisfaction with the number of administrations per day of the medication, and satisfaction with the caregiver own knowledge about the treatment of diabetes). Each of the 8 items are scored on a descending scale of five levels: Very satisfied / Fairly satisfied / Normal / Fairly dissatisfied / Very dissatisfied. This questionnaire was administered to a cohort of 79 caregivers. The internal consistency reliability, as estimated by Cronbach's alpha coefficient, was 0.85, indicating that the components of the scale measured the same construct. The items all significantly correlated with each other ($r = 0.21$ to 0.80 , $P < 0.001$). Stronger correlations of global satisfaction were demonstrated with satisfaction with the 'blood sugar levels' ($r = 0.74$, $P < 0.001$), with satisfaction in continuing with the current treatment ($r = 0.62$, $P < 0.001$), and with the willingness to recommend the current treatment to other people ($r = 0.62$, $P < 0.001$). Global satisfaction with treatment ($r = -0.43$, $P < 0.001$) and satisfaction with the glycaemic levels ($r = -0.57$, $P < 0.001$) showed the higher correlations with HbA_{1c}. Factor analysis identified two components explaining 72.1% of the variance. The intraclass correlation coefficient between scores on both administrations of the questionnaire was 0.89.

Conclusion: The STCD₂ questionnaire is a very easy to administer, reliable, consistent and valid instrument for measuring satisfaction with the treatment in caregivers of dependent type 2 diabetic patients.

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Executive functioning in type 2 diabetes: results from the LifeLines cohort Study

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Background and aims: People with diabetes are at increased risk of cognitive dysfunction. We investigated executive function in people with self-reported and newly-diagnosed type 2 diabetes, and compared results with non-diabetic individuals, as well as the relation with metabolic control and anthropometric measures.

Materials and methods: We studied 10,024 participants aged 30 years and above of the population-based LifeLines Cohort Study. Neuropsychological test scores were examined in one cognitive domain, i.e. executive function

with the Ruff's Figural Fluency Test (sum of unique patterns) and expressed as standardized Z-values. Diabetes was defined as self-reported ($n=226$), and newly-diagnosed by fasting blood glucose ≥ 7.0 mmol/l at screening ($n=120$). Data were analyzed using Z-scores adjusted for age with Pearson's correlation and multiple linear regression.

Results: Mean age was 49 (SD 11, range 31–88) yrs; 58% was female. RFFT score was 81 ± 22 in the non-diabetic individuals, 72 ± 23 in the newly-diagnosed group, and 68 ± 21 in the known diabetes group ($p < 0.001$). Mean age differed between groups, and was lower in the controls: 49 ± 11 , 56 ± 11 , and 59 ± 11 yrs, respectively. Compared to the controls, Z-score of RFFT was -0.22 (0.97) in the known diabetics ($p=0.001$) and -0.14 (1.06) in the newly-diagnosed ($p=0.12$). Log-transformed fasting blood glucose ($r=-0.15$), HbA1c ($r=-0.17$), age ($r=-0.37$), BMI ($r=-0.11$) and WHR ($r=-0.13$) correlated significantly (all $p < 0.001$) with RFFT-score. In multiple regression analysis, age, HbA1c, BMI and WHR proved to be independent predictors of RFFT-score.

Conclusion: Diabetes and level of glycaemic control, as well as anthropometric measures, are associated with slightly worse cognitive performance in executive function after adjustment for age.

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The impact of glucose lowering agents on treatment satisfaction and psychological well-being in patients with type 2 diabetes

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Background and aims: Diabetes Mellitus (DM) requires a high level of self-care and commitment from the patient. Perception of different glucose lowering alternatives and their effects on general well-being is not well explored in patients with DM. The objective of this study was to determine if initiation of insulin influenced treatment satisfaction and mental well-being in such patients.

Materials and methods: In the DIGAMI 2 study patients with diabetes and myocardial infarction were randomised to insulin based treatment or treatment based on oral drugs. Information at admission and after 12 months of follow-up was available in 324 patients (median age 67 years, interquartile range 59–73; 71% men). They were evaluated by means of the Diabetes Treatment Satisfaction Questionnaire (DTSQ) and the Psychological General Well-being index (PGWB) at baseline and after 12 months. Patients with insulin-based glucose control ($n=197$) were compared to those on oral glucose lowering agents ($n=127$).

Results: There was no difference in age and gender between groups. At baseline insulin treated patients had a worse risk profile, more co-morbidities and were less satisfied with treatment (DTSQ 28.6 ± 6.5) compared to patients on oral treatment (DTSQ 30.6 ± 5.1 ; $p=0.019$). Treatment satisfaction improved significantly after 12 months in both groups and there was no longer a difference in DTSQ score between patients on insulin (30.9 ± 5.7) and oral treatment (31.8 ± 5.3 , NS). This pattern was the same in patients not previously treated with insulin but randomised to such treatment (DTSQ 31.8 ± 5.0 vs. 31.8 ± 5.3 , NS). PGWB scores were similar in the insulin and oral treatment groups at baseline (77.2 ± 6.7 vs. 78.1 ± 6.1) and after 12 months (80.2 ± 5.2 vs. 80.5 ± 5.6). The improvement in PGWB was significant in both groups. Men had higher DTSQ and PGWB scores compared with women at baseline and after 12 months. Both genders improved DTSQ and PGWB significantly over time, independent of glucose lowering treatment.

Conclusion: Insulin based glucose lowering treatment did not affect treatment satisfaction or psychological well-being negatively in patients with type 2 diabetes and myocardial infarction compared with their orally treated counterparts.

Clinical Trial Registration Number: 96-164

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Is hypoglycaemia worrisome even for diet-only treated people with type 2 diabetes?

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Background and aims: Hypoglycaemia is a frequent experience in people with insulin-treated diabetes mellitus. It is presumed to be much less frequent

in OAD-treated patients and virtually non-existent in diet-only treated patients, in daily practice however, the latter two patient groups still express their worry about hypoglycaemia, which may often interfere with decision-making about the therapy up-grade and the overall success of diabetes treatment. The aim of our study was to explore to what extent do OAD and diet-only treated patients worry about hypoglycaemia.

Materials and methods: In this retrospective study, we looked at patient charts of all 611 OAD and diet-only treated patients (diabetes educated at some point), who visited our centre at least once in year 2010. Those who completed all items of the 20-item Problem Areas In Diabetes (PAID) questionnaire, as part of a routine first visit in the year, qualified for our survey: 294 completers on OAD and 141 completers on diet-only treatment (further 100 refused to complete; 76 partly completed). We looked at the question No.9: 'Worrying about low blood sugar reactions?' If they at least marked 'Minor problem' (or more), they classified as worrying about hypoglycaemia (PAID yes), if hypoglycaemia was mentioned in the chart at any time before this visit, they classified as experiencing at least one episode (Hypo yes). Sulphonylurea (SU) use(%) was added to the OAD scoring results, as it carries most risk for hypoglycaemia. Descriptive statistics was used to present the data.

Results: Patient characteristics were: 141 on diet-only (83 males; 59%), avg. (SD; range) age 61y.(10;30–82), diabetes duration 4.2y. (5.5;0–27), HbA1c 6.8% (1.2;4.7–13.8) and 294 on OAD (168 males;57%), age 62y.(11;32–89), diabetes duration 7.8(7.3;0–40), HbA1c 7.3% (1.23;5.2–12.9). Number of subjects scoring PAID and experience of hypoglycaemia, along with SU use is presented in the Table.

Conclusion: In diet-only treated patients roughly half worry about hypoglycaemia. In OAD-treated patients almost 60% worry about hypoglycaemia, among those, only one quarter actually experienced an episode; in those that did not have an episode, roughly half are those that worry. Within the OAD-treated group SU seems to play little role in understanding why people worry about hypoglycaemia or not. Worry about hypoglycaemia goes beyond the experience of hypoglycaemia, treatment mode or knowledge about diabetes, therefore other factors remain to be elucidated.

		PAID diet - only				PAID OAD	
		yes	no			yes	no
hypo	yes	2 (1,4 %)	1 (0,7 %)	hypo	yes	40 (13,6 %)	11 (3,7 %)
	no	67 (47,5 %)	71 (50,4 %)		no	SU 70 %	SU 82 %
SUM		69 (48,9 %)	72 (51,1 %)	SUM		130 (44,2 %)	113 (38,4 %)
						SU 71 %	SU 64 %
						170 (57,8 %)	124 (42,2 %)
						SU 71 %	SU 65 %

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Psychoemotional implications of anxiety and depression on the eating behaviour of newly diagnosed type 2 diabetes patients

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Background and aims: Numerous studies have reported high prevalence of eating disorders among patients with type 1 diabetes mellitus, but with little evidence of the association of these disorders in patients with type 2 diabetes that associate anxiety and depression. Our aim was to establish the prevalence of anxiety, depression and eating disorders such as bulimia nervosa and binge eating disorder among patients with newly diagnosed type 2 diabetes mellitus and the implications of psychoemotional status on eating behavior.

Materials and methods: We developed a cross-sectional study, descriptive, over 80 patients with newly diagnosed type 2 diabetes mellitus who were administered standardized instruments for assessing eating disorders (Eating Attitudes Test-26), depression and anxiety (Hamilton Anxiety Scale and Hamilton Depression Scale). We have also compiled a questionnaire which includes demographic data, information on socio-professional status and socio-economic status, coronary risk factors, medical history, family history, any medication, eating habits and we've adapted various items of standardized tests for assessment of eating disorders (Eating Disorder Diagnostic Scale, Eating Disorder Examination, Eating Behavior Severity Scale). Associated we used diagnostic criteria for eating disorders from the Diagnostic Statistical Manual IV. After assessing the prevalence of bulimia nervosa and binge eating disorder in the newly diagnosed type 2 diabetic patients, we divided the

study population into two sub-groups: newly discovered type 2 diabetic patients with and without an eating disorder.

Results: 23.7% of patients with newly diagnosed type 2 diabetes associated bulimia nervosa or binge eating disorder. 38.75% of patients showed varying degrees of anxiety (48.3% mild anxiety, 42% intermediate and 9.7% severe anxiety), without statistically significant differences between patients with and without eating disorder; prevalence of depression was 21.2%, significantly higher among people with diabetes who associated an eating disorder ($p < 0.01$). High depression scores were associated with a higher daily frequency of meals and snacks and increased consumption of farinaceous products, sweets and pastries ($p < 0.01$), while patients who have elevated anxiety scores tended to limit the number of daily snacks and avoid these foods ($p < 0.01$). Glycated hemoglobin values were inversely correlated with the degree of anxiety and directly proportional correlated with depressive symptoms ($p < 0.01$). Disappointment, sadness, concern, irritability are strongly and statistically significantly related with high scores in Eating Attitudes Test-26, body mass index and glycated hemoglobin values ($p < 0.01$).

Conclusion: Binge eating disorder was the most frequent type of eating disorder associated with type 2 diabetes mellitus at onset. Psychoemotional context, anxiety and depression are related with eating behavior of newly diagnosed type 2 diabetic patients. Unlike anxious patients, depressed subjects tend to have more daily snacks and meals and a higher consumption of hypercaloric foods. Depression and eating disorders prone to a poorer glycemic profile, while glycated hemoglobin values of anxious patients proved a better metabolic profile.

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Illness intrusiveness and the longitudinal prediction of depression in adults with newly diagnosed type 2 diabetes mellitus

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Background and aims: Although the risk of depression is low in people newly diagnosed with type 2 diabetes mellitus (T2DM), this risk increases over the first year after diagnosis. It is likely that the burden of living with diabetes and having to care for it contribute to the development of depressive symptoms. Illness intrusiveness (the perception that the illness and its ramifications disrupt valued daily activities) is associated with increased depressive symptoms in people with longstanding T2DM. Whether illness intrusiveness is a mere correlate of depression or whether it contributes to its development remains to be determined. Thus, research is needed to examine the longitudinal relationship between perceptions of the impact of diabetes and depressive symptoms during the critical period after diagnosis. It is also possible that depression occurs when people feel that they are unable to attain important life goals. To date, this has not been examined in relation to T2DM. Therefore, we examined the longitudinal relationship between perceptions of illness intrusiveness, goal self-efficacy and depressive symptoms in a sample of newly diagnosed people with T2DM.

Materials and methods: 237 people (65% male) newly diagnosed with T2DM completed questionnaires assessing depressive symptoms (BDI-short), perceptions of illness intrusiveness, and number of diabetes-related complications, on five occasions, each three months apart. On each occasion, we also assessed perceived ability to attain key life goals (goal self-efficacy) using semi-structured interviews, along with BMI and HbA1c.

Results: Over the 18-month study period, the number of people with significant depressive symptoms (BDI-short > 9) ranged from 38 (16%) at Time 1 to 22 (11%) at Time 5. Multivariable Generalised Estimating Equations (GEE) analyses, showed that both goal self-efficacy ($\beta = -0.09$, $p < 0.02$) and illness intrusiveness ($\beta = 0.20$, $p < 0.001$) were significant longitudinal predictors of depressive symptoms, even when controlling for age, sex and BMI. HbA1c and self-reported complications were not significantly associated with depressive symptoms. When changes between two adjacent time points were modeled, multivariable GEE analyses showed that changes in depressive symptoms were predicted by changes in perceived illness intrusiveness ($\beta = 0.16$, $p < 0.001$) but not by changes in goal self-efficacy or HbA1c.

Conclusion: Depressive symptoms were associated with illness intrusiveness and goal self-efficacy. However, diabetes control and self-reported complications did not contribute to the prediction of depressive symptoms over time. These results reflect a combination of cross-sectional and longitudinal associations. Changes in depressive symptoms were predicted by changes in illness intrusiveness only and not by changes in goal self-efficacy, diabetes control or complications. Overall, the results show that people who doubt

their ability to attain important life goals are more depressed. When people perceive changes in diabetes-induced disruptions of activities between time points, their depressive symptoms changed accordingly. To reduce depression in people with newly diagnosed T2DM, interventions should target perceived conflicts between valued activities and diabetes self-care activities.

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Should we expect gender differences in reporting of depressive symptoms and seeking of professional help among diabetic patients?

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Background and aims: Research data have shown that depressive symptoms are prevalent in persons with diabetes. Women with diabetes exhibit higher rates of depressed mood than diabetic men. Data from the general population indicate that women are more likely to seek professional help for mental health. However, research on the differences in seeking help for depression between diabetic men and women is scarce. This study was aimed at examining gender differences in responding to depression screening, reporting depressive symptoms and expressing a need for professional help in a large sample of Croatian patients with diabetes.

Materials and methods: One thousand and six hundred type 2 diabetic patients were selected from the database of patients treated at a tertiary diabetes clinic in an urban setting. They were screened for depressive symptoms by using the two-item Patient Health Questionnaire-Depression (PHQ-2) accompanied by an additional question inquiring into their need to receive professional help. The questionnaire and an envelope for a pre-paid reply was sent to the recipients by surface mail. Demographic and disease-related data were collected from patients' medical records. Response rate, prevalence of depressive symptoms and the need for professional help were compared in female versus male respondents by using chi-square test. Disease-related characteristics were compared between depressed and non-depressed groups by t-test.

Results: The response rate of 42% was obtained, yielding a sample size of $n = 674$ (49% female, aged 62 ± 2 years, with HbA1c of 7.1 ± 1.3 , and BMI of $29.9 \text{ kg/m}^2 \pm 4.7$). Forty-seven percent of the responders reported having experienced at least one of the depressive symptoms, while 35% reported both symptoms. Of the responders, 45% reported low mood and 37% a lack of interest and pleasure. A need for receiving professional help was expressed by 78% patients burdened by depressive symptoms. No gender differences were observed in either the response rate (chi-square=0.02, $p = 0.90$), the presence of depressive symptoms (chi-square=0.35, $p = 0.84$) or the expressed need to receive professional help (chi-square=0.04, $p = 0.85$). Patients with depressive symptoms had a higher BMI than those who were symptom-free (30.2 ± 4.9 vs 29.3 ± 4.5 , $p = 0.03$). HbA1c values were shown to be nearly significantly higher in patients with elevated depressive symptoms ($7.2\% \pm 1.3$ vs $7\% \pm 1.3$, $p = 0.07$) whereas other metabolic parameters, including total cholesterol, triglycerides, LDL, HDL and albumin/creatinine ratio did not differ between the groups (all p 's $> .05$).

Conclusion: Unexpectedly, no differences were found between women and men with diabetes in responding to depression screening, reporting depressive symptoms and expressing a need for professional help. Further research is needed to clarify whether these data are due to self-selection inherent to the screening process or they reflect specific features in persons with diabetes.

Clinical Trial Registration Number: ISRCTN05673017

Supported by: an EFSN New Horizons grant

PS 083 Metabolic self monitoring

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Self-monitoring of glucose in blood or urine does not increase diabetes-specific distress in non-insulin treated patients with type 2 diabetes: results from the IN CONTROL-trial

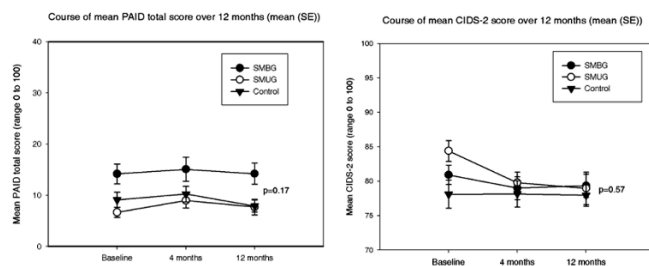
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Background and aims: Self-monitoring of glucose is widely considered as an important tool in diabetes self-management. Concerns about a possible negative impact of self-monitoring on quality of life and self-care behaviours have risen. We investigated the effects of self-monitoring of glucose in blood or urine, on diabetes specific distress and self-efficacy, compared to usual care in non-insulin treated patients with type 2 diabetes.

Materials and methods: We recruited 181 patients from 3 diabetes care systems with type 2 diabetes treated with diet or oral hypoglycaemic agents, (mean (SD) HbA1c of 58 (15) mmol/mol, mean age 61.5 (7.8) years, median (q1, q3) diabetes duration of 5 (3, 9) years) and randomised them to either SMBG (pre- and post-prandial testing around main meals, two separate days a week) (n=60), SMUG (post-prandial testing after dinner, two separate days a week) (n=59) or Usual Care (n=62). All patients received standardized diabetes care with education. The intervention groups were provided with a flowchart and instructions how to interpret self-monitoring results and what actions to take. Self-administered questionnaires were completed before randomisation at baseline, 4 and 12 months follow-up. Intention-to-treat (ITT) analyses were performed. Primary outcome measures were between group differences in diabetes specific distress (PAID; range 0 to 100) and self-efficacy (CIDS-2; range 0 to 100) after 12 months. Cut off points were 40 for PAID, indicating high distress and 71.5 for CIDS-2 indicating high self-efficacy.

Results: There were no statistically significant between-group differences in changes in PAID ($p=0.17$) and CIDS-2 ($p=0.54$) after 12 months. Mean difference in PAID over 12 months between SMBG and Usual Care, adjusted for baseline levels was 2.56 points (95% CI -0.12 to 5.24) and between SMUG and Usual care 0.81 points (95% CI -2.10 to 3.72). Mean difference in CIDS-2 over 12 months between SMBG and usual care, adjusted for baseline levels was 0.49 points (95% CI -2.65 to 3.64) and between SMUG and Usual Care -1.43 points (95% CI -4.95 to 2.09).

Conclusion: The study demonstrated that self-monitoring of glucose in blood or urine did not promote diabetes related distress and suggest that self-monitoring had no clinical relevant impact on diabetes specific quality of life in moderately controlled patients with type 2 diabetes treated with diet or oral hypoglycaemic agents. Figure 1: Course of mean PAID and CIDS-2 scores over 12 months (High scores indicate high distress / self-efficacy)



Clinical Trial Registration Number: ISRCTN 84568563
Supported by: an EFSD grant

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Self-monitoring of glucose levels in blood or urine does not change illness beliefs about control: results from the IN CONTROL-trial

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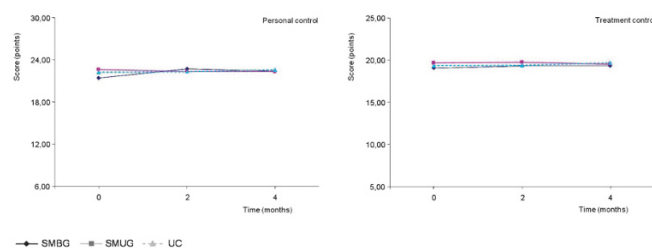
Background and aims: The effectiveness of self-monitoring of glucose in non-insulin requiring type 2 diabetic patients has been under discussion for years. The IN CONTROL-trial explored whether self-monitoring can affect diabetes specific emotional distress and self-efficacy in these patients. The hypothesis was based on the common sense model and supposed that feedback from self-monitoring allows adaptation of illness perceptions, which supported by adaptations of perceived social support, mediate changes in distress and self-efficacy. To explore the theory underlying the effects of self-monitoring, this study analyzed whether self-monitoring of glucose changed illness perceptions at short-term.

Materials and methods: The 181 participants of the IN CONTROL-trial, recruited from three diabetes care systems, were randomized to the intervention groups self-monitoring of blood glucose (SMBG, n=60) and self-monitoring of urine glucose (SMUG n=59), and Usual Care group (UC, n=62). Participants in the intervention groups received a training and a flowchart with instructions, and all participants received standard diabetes care with education. They completed at baseline, two and four months after baseline self-reporting questionnaires assessing illness beliefs with the revised Illness Perception Questionnaire (IPQ-R) and the Multidimensional Diabetes Questionnaire (MDQ). The domains "personal control" (score range 6 to 30) and "treatment control" (score range 5 to 25) of the IPQ-R, and "perceived social support" and "perceived severity" of the MDQ (score range 0 to 6) were used. Higher scores indicate higher self-reported experience of "control", "social support" or "severity". Change scores after two and after four months of the four subscales were calculated and compared between SMBG and Usual Care groups, SMUG and Usual Care groups and SMBG and SMUG groups. A complete case analysis was performed as subgroup analysis.

Results: All illness perception questionnaire and multidimensional diabetes questionnaire change scores after two months were small (range from -0.36 to 1.16) and not statistically significant. After four months all change scores were small as well (range from -0.47 to 0.98) and not statistically significant different between the three groups.

Conclusion: This study demonstrated that self-monitoring of glucose levels in blood or urine did not change illness perceptions after two and after four months.

Figure 1: Course of mean "personal control" and "treatment control" scores (complete cases)



Clinical Trial Registration Number: ISRCTN 84568563
Supported by: an EFSD grant

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Postprandial urine tests for glucose: a reliable, painless and inexpensive monitoring of metabolic control in type 2 diabetes without insulin treatment

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Background and aims: Over 100 years ago A. Bouchardat, and, subsequently, E. P. Joslin trained people with diabetes to test their urine for glucose. Postprandial urine tests for glucose are promoted as a method for self monitoring in type 2 diabetes without insulin treatment. Nevertheless, the relation be-

tween the results of postprandial measurements of glucosuria by the patients and HbA1c levels has yet to be studied systematically. Blood glucose monitoring is over 10-times more costly and heavily promoted. Therefore, the aim of this study was to evaluate the association between postprandial glucosuria and HbA1c. Our hypothesis being that urine testing for glucose is a reliable method for identifying a good level of metabolic control.

Materials and methods: Data from 216 patients (47.7 % female) with diabetes mellitus type 2 without insulin treatment were analysed (avg. age 62.3 years, time since diagnosis of diabetes 7.0 years, BMI 30.7 kg/m², HbA1c 6.6 %, 25% were treated with diet without oral antidiabetic agents). The patients had been recruited within the frame of two cross-sectional studies in a university clinic for endocrinology and metabolic diseases and three practices for general medicine. The patients' log books were analysed to check if patients had measured glucosuria at least once per week. The resulting urine tests during the final four weeks were considered for the study. 85.2 % of the patients had previously participated in a structured education programme. According to the results of urine testing for glucose, the patients were divided into three groups: Urine tests always negative, < 50 % of tests positive and ≥ 50 positive. HbA_{1c} results were adjusted to the DCCT standard.

Results: The patients performed 9.8 per urine tests per week (mean, range 1–21). The urine tests were always negative in 57.4% of the patients (HbA1c 6.3 %, min 5.0, max 7.9%; age 63.5 years, time since diagnosis 6.1 years, BMI 31.7). 30.6 % had <50% positive results testing for glucosuria (HbA1c 6.8 %, min 5.5, max. 9.0 %, age 60.9 years, 8.9 years since diagnosis, BMI 29.4) and 12.0% had ≥50% positive result (HbA1c 7.4%, min 6.2, max 9.1%, age 59.7 years, time since diagnosis 6.6 years, BMI 29.5). The frequency of positive SMUG tests had a significant positive correlation with HbA1c ($R^2 = 0.265$, $p < 0.001$).

Conclusion: Patients with diabetes type 2 and without insulin treatment who are negative for postprandial glucosuria have a near normal HbA1c range. There was a strong statistical relationship between the results of urine testing for glucose and HbA1c. Postprandial testing urine tests for glucose are an effective, painless and inexpensive method for metabolic self-monitoring in patients with type 2 diabetes without insulin therapy.

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Episodic, intensive SMBG in non-insulin-treated T2DM: can this form of patient-provided data be trusted?

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Background and aims: In anticipation of an upcoming medical visit, T2DM patients may temporarily “flee to health”, choosing to adhere more closely to medical recommendations. Therefore, one concern about the use of patient-provided SMBG data, especially if the data are restricted to only the few days prior to the appointment, is that these data may not be truly representative of the patient's glycemic status. Given the growing interest in intensive, episodic approaches to SMBG, this is a critical issue, since these data may be used to guide treatment recommendations. How concerned should we be?

Materials and methods: To address this question, we examined data from the Structured Testing Program (STeP), which investigated the impact of structured SMBG use on HbA1c over 12 months. In this prospective, cluster-randomized, multi-centered clinical trial, 483 poorly-controlled (HbA1c ≥7.5%), non-insulin-treated T2DM patients were randomized to structured testing (STG) or active control (ACG). STG subjects used the ACCU-CHEK® 360° View Blood Glucose Analysis System to collect/interpret 7-point glucose profiles over each of 3 consecutive days. They then brought the data to be reviewed with their physician. In this report, we focus only on STG patients ($n = 227$), who completed the tool on a quarterly basis (at months 1, 3, 6, 9 and 12) and shared the results with their physician. HbA1c was also collected at each quarterly visit. STG patients and physicians received standardized instruction in SMBG pattern recognition/interpretation. All patients received free blood glucose meters and test strips.

Results: At each quarterly visit, mean patient-reported blood glucose levels across the 3-day record were highly correlated with HbA1c (month 1, $r = .72$; month 3, $r = .77$; month 6, $r = .68$; month 9, $r = .70$; month 12, $r = .76$; in all cases, $p < 0.0001$). In addition, change in mean, quarterly patient-reported blood glucose levels was significantly associated with change in quarterly HbA1c over the entire 12 months ($r = .75$; $p < 0.0001$). The correlations were not significantly different across age, gender, ethnicity, BMI or diabetes duration.

Conclusion: Intensive, episodic, patient-reported SMBG data, covering three days prior to a quarterly medical visit, are significantly related to overall glycemic control, as assessed by HbA1c. Furthermore, change in these mean episodic SMBG data over 12 months is closely associated with change in HbA1c over the 12 months. These findings suggest that intensive SMBG data accurately reflect overall glycemic control and can be used to adjust treatment without fear of a “flight to health” effect. Indeed, as we have reported previously, the use of such data in the STeP study was found to be associated with a significantly greater long-term glycemic improvement than found among control subjects.

Clinical Trial Registration Number: NCT00674986

Supported by: Roche Diagnostics, Inc.

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Long-term effects of self-monitoring of blood glucose on glucometabolic control in patients with type 2 diabetes mellitus: follow up data from ROSSO-in-praxi international

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Background and aims: As essential risk factors for type 2 diabetes mellitus (T2DM) are obesity, lack of physical activity and high caloric diet, the basis for all therapies should be a lifestyle change. Self-monitoring of blood glucose (SMBG) is a simple tool to visualize the effects of lifestyle change on blood glucose levels in insulin-naïve patients with T2DM. Recently, we could demonstrate by the randomized controlled trial ‘ROSSO-in-praxi international’ that a 12-week SMBG-structured lifestyle intervention improves glucometabolic control of T2DM patients. So far it is unknown if such a short-time intervention also has long-term effects. Therefore, patients were followed for a mean period of 1.5 years.

Materials and methods: 124 SMBG-naïve T2DM ambulatory patients had been randomly assigned to a SMBG ($n=63$) and a control group ($n=61$). Both groups got a manual with basic information about healthy lifestyle. Glucometabolic parameters were assessed at baseline, after 12 weeks and again after 1.5 years and compared using Friedman test + Dunn's multiple comparison test.

Results: During the 12 weeks of intervention participants in the SMBG group significantly improved their HbA1c (from $7.4 \pm 1.6\%$ to $6.9 \pm 1.1\%$; i.e. -0.5% ; $p < 0.001$) and lost weight (-1.0 kg; $p < 0.05$), while HbA1c reduction (from $7.5 \pm 1.0\%$ to $7.3 \pm 1.0\%$; i.e. -0.2%) and weight loss (-0.6 kg) were not significant in the control group. In addition, cardiovascular risk factors such as waist circumference, systolic and diastolic blood pressure, total and LDL cholesterol as well as triglyceride levels significantly improved just in the SMBG group. Patients were followed up for a mean of 1.5 years with 122 out of 124 patients completing the follow up. Interestingly, HbA1c level increased again in the control group, reaching baseline values (i.e. $7.5 \pm 0.7\%$). In the SMBG group improvement of HbA1c ($6.9 \pm 0.9\%$; i.e. -0.5% vs. baseline; $p=0.0003$ for trend) as well as systolic and diastolic blood pressure and lipid parameters maintained stable, while weight (-1.7 kg vs. baseline; $p=0.0003$ for trend), BMI and waist circumference improved further. 87% of participants in the SMBG group continued to perform SMBG. Those who measured their blood glucose $>3x$ per week, demonstrated an overall reduction in HbA1c of 1.0% ($p=0.006$ vs. those who measured at most $3x$ per week) after 1.5 years.

Conclusion: Integration of a short-time, highly motivational and low-cost intervention into basic therapy of T2DM improves glucometabolic health and has long-term effects on glucometabolic control. SMBG might be a motivational tool for lifestyle change. But any benefit will depend on the ability of patients to understand the results and to respond appropriately. Clearly, SMBG may not be useful to patients who lack the necessary skills and motivation for proper documentation and utilization of SMBG results. Therefore, SMBG should be offered to all T2DM patients who are trying to adapt to a healthier lifestyle.

Supported by: Roche Diagnostics Deutschland GmbH.

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Evidence of a strong association between the frequency of self-monitoring of blood glucose and haemoglobin A_{1c} levels in type 1 diabetes exchange participants

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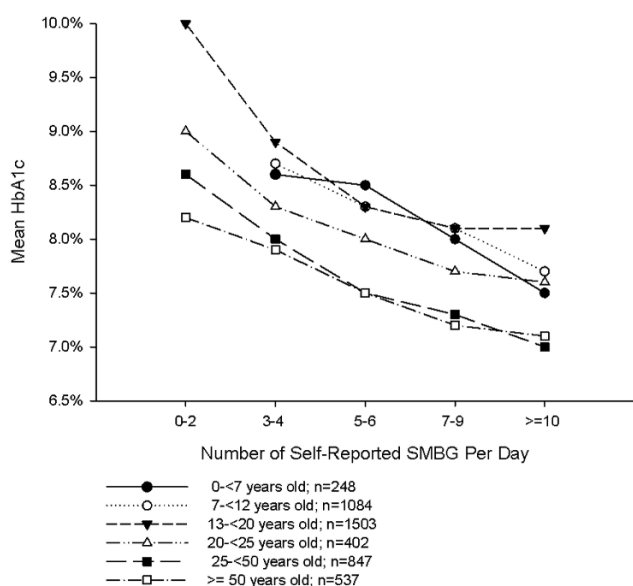
Background and aims: American insurance companies have questioned whether or not sufficient evidence is available to justify unlimited coverage of Self-Monitoring of Blood Glucose (SMBG) test strips for patients with type 1 diabetes (T1D). The large database of the T1D Exchange clinic registry provided an opportunity to evaluate the relationship between the number of times SMBG is performed per day and Hemoglobin A_{1c} (HbA_{1c}) levels across a wide age range of children and adults.

Materials and methods: The registry, which currently includes over 50 centers in the U.S., commenced enrollment of children and adults with T1D in September 2010, with longitudinal participant data collected through medical records and participant or parent/guardian of participant (for child participants) questionnaires. SMBG frequency was self reported by 4621 participants who were not using a real-time continuous glucose monitor (CGM) (age range 0 to 91 years, 51% female, 86% non-Hispanic white, mean diabetes duration 11.4 years, 54% using a pump), of whom downloaded meter data were available for 2523 (55%). The analysis assessing the association between SMBG and mean HbA_{1c} was stratified by age group and separately by insulin method (insulin pump and injection) for child (< 18 years old) and adult participants. The 854 participants using CGM were not included in the analyses.

Results: Among the 4621 participants the mean HbA_{1c} was $8.2\% \pm 1.5\%$ and the mean self reported frequency of SMBG measurements was 6 ± 2 per day. Among the 2523 participants with both self reported and downloaded data, the mean self-reported SMBG was 6 ± 2 , where the SMBG from meter downloaded data was 5 ± 3 . A strong association ($P < 0.001$) was present between frequency of SMBG measurements and HbA_{1c} levels, with increasing daily self-reported SMBG frequency being associated with lower HbA_{1c} levels for all age groups [Figure 1]. The same relationship was observed when the analysis was stratified by pump verse injection users separately for child (< 18 years) and adult participants ($P < 0.001$ for all comparisons). Similar strong associations were seen when the meter downloads were evaluated.

Conclusion: Across a wide age range of children and adults, there was a strong linear relationship in which higher daily frequencies of SMBG measurements were associated with lower HbA_{1c} levels. A similar relationship between frequency of SMBG and HbA_{1c} was observed for both injection and pump users for child and adult participants.

Figure 1. Frequency of SMBG and Mean HbA_{1c} by Age Group



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Decision support tools improve clinicians' ability to interpret structured SMBG data

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Background and aims: Self-monitoring of blood glucose (SMBG) is useful only when the glucose information is collected in a structured manner, the data are accurately interpreted, and the results prompt appropriate therapeutic actions. The Accu-Chek® 360° View blood glucose analysis system is a simple paper tool that facilitates collection and visualization of 7-point glucose profiles from 3 consecutive days. Using this tool, 78% of clinicians were able to correctly identify the primary glycemic abnormalities. However, only 66.4% of clinicians correctly identified fasting/preprandial hyperglycemia. Utilizing the 360° View tool in a large, cluster-randomized, clinical trial, the STeP study demonstrated significant reductions in HbA_{1c} when structured SMBG is combined with comprehensive clinician education regarding data interpretation and use. We developed an automated decision support tool (DST) for use with the 360° View form. The DST algorithm analyzes SMBG data from the 360° form and generates a printed report that identifies the primary glycemic abnormality and recommends appropriate therapeutic options. We evaluated the impact of the DST on clinicians' ability to identify glycemic abnormalities in SMBG data.

Materials and methods: In this prospective, randomized, controlled, multicenter study, 288 clinicians (39.6% family practice physicians, 37.9% internal medicine physicians, and 22.6% nurse practitioners) were randomized to four study groups: structured SMBG alone (Group A, n=72); structured SMBG with DST (Group B, n=72); structured SMBG with educational DVD (Group C, n=72); and structured SMBG with DST and educational DVD (Group D, n=72). Clinicians were asked to analyze 30 patient cases drawn from the STeP study cohort, identify the primary abnormality, and select the most appropriate class of drug to treat the abnormality. An expert clinician panel reviewed the cases and established correct answers.

Results: 223 clinicians completed all 30 patient cases with no major protocol deviations. Significantly more Group D clinicians (87%) correctly identified the primary glycemic abnormalities than Group A clinicians (51%, $p < 0.0001$), Group B clinicians (77%, $p = 0.0454$) and Group C clinicians (72%, $p < 0.0001$) (Figure 1). Significantly more Group D clinicians (86%) accurately identified preprandial glycemic abnormalities than clinicians in Groups A (47%, $p < 0.0001$) and C (64%, $p < 0.0001$); Groups B and D were not significantly different. Significantly more Group D clinicians (86%) accurately identified postprandial glycemic abnormalities than clinicians in Groups A (55%, $p < 0.0001$) and B (73%, $p = 0.027$); Groups C and D were not significantly different.

Conclusion: The recent STeP study demonstrated the clinical efficacy of structured SMBG when combined with comprehensive clinician education. Addition of the new automated decision support technology (DST) significantly improved the clinicians' ability to accurately interpret structured SMBG data.

Supported by: Roche Diagnostics, Inc.

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Association of self monitoring of blood glucose, medication adherence, and glycaemic control in diabetes patients not using insulin

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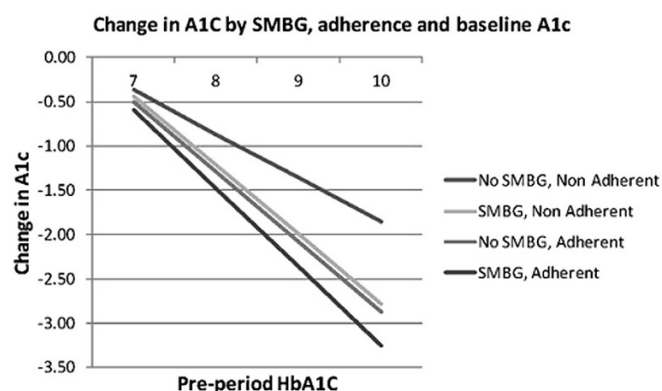
Background and aims: There is debate about the value of SMBG in non-insulin type 2 diabetes necessitating further research. The aim of this study was to examine the associations between SMBG presence, medication adherence, and improved glycemic control.

Materials and methods: Medication naïve type 2 diabetes patients, 18-63 years of age, who began non-insulin diabetes therapy (including injectables) between 1 October 2006 through 31 March 2009 were identified using a US commercial insurance claims database (i3 Innovus). Patients were required to have A1C values within the 3 months before and 4-12 months after medica-

tion initiation. A medication possession ratio (MPR) was calculated for each patient. Patients were considered adherent if the MPR $\geq 80\%$. Logistic regression was used to calculate odds ratios for medication adherence. ANCOVA was performed to assess for interactions between SMBG presence, A1c, and medication adherence.

Results: In the 5172 patients, initiation therapies were metformin (69%), sulfonylurea (10%), TZDs (10%), or DDP-4 inhibitors (6%). 2,744 patients (53.1%) had SMBG available post-medication start and 2,428 (46.9%) did not. SMBG presence was associated with a higher likelihood than SMBG absence of being medication adherent (OR 1.5, 95% CI 1.3–1.7). Medication adherent patients had a significantly larger predicted decline in A1c in the post-medication period (-1.35 vs. -0.82 for non-adherent patients, $p < 0.001$). ANCOVA controlling for age, gender, and prior A1c demonstrated a significant interaction among SMBG presence, medication adherence, and baseline A1c ($p < 0.0001$). Higher baseline A1c was associated with greater decline in A1c, post-medication. Both SMBG presence and medication adherence were associated with a similar degree of decrease in A1c. For example, A1c is 9% and other factors are held at mean values, among nonadherent patients the change in A1c was -2.00 for SMBG and -1.36 ($p < 0.0001$) for no SMBG; and for medication adherent patients, -2.37 for SMBG and -2.08 for no SMBG ($p < 0.0001$).

Conclusion: SMBG presence was associated with greater medication adherence. Additionally, SMBG presence was associated with improvement in glycemic control for both medication adherent and non adherent patients. This suggests an association of SMBG presence with glycemic control independent of medication adherence.



PS 084 Continuous glucose monitoring

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Comparison of patient-led or physician-driven continuous glucose monitoring in poorly-controlled type 1 diabetic patients: a one-year multicenter study

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Background and aims: Benefits of real-time continuous glucose monitoring (CGM) have been shown in type 1 diabetic (T1D) patients in 3- to 6-month studies. The aim of the present study is to assess the effect of two approaches of one-year use of CGM in poorly-controlled T1D

Materials and methods: The study protocol was designed as a 1-year, multicenter trial. Inclusion criteria were age ≥ 8 yrs, T1D for ≥ 1 year, use of either multiple daily insulin injections (MDI) or an insulin pump and A1c level $\geq 8\%$. Patients were randomly assigned into 3 groups (1:1:1). Two modes of using CGM (FreeStyle Navigator™) were considered: Group 1 (G1): patient-led, Group 2 (G2): physician-driven where sensors were prescribed initially 50% of the time and more often if the targets were not reached (A1c $< 7.5\%$, < 4 mild hypoglycaemia/week, no severe hypoglycaemia). These two strategies were compared to a Self Blood Glucose Monitoring practice (Group 3 (G3): control). The primary outcome was the change in A1c level at one year.

Results: Overall, 178 patients completed the study: age 36 ± 14 yrs, duration of T1D: 17 ± 10 yrs, A1c: $8.9 \pm 0.9\%$, SD glucose (8-point blood glucose profile): $70 [52; 84]$ mg/dl (mean \pm SD or median [95% CI]). At one year, A1c changes were similar in both CGM groups, and significantly higher than in the control group: G1 vs G3: -0.52% , $p = 0.0006$, G2 vs G3: -0.47% , $p = 0.0008$, G1+G2 vs G3: -0.50% , $p < 0.0001$. SD glucose was reduced only in group 2 by $15.7 [-28.8; -4.61]$ vs G3 by $0.6 [-8.9; -4.6]$ mg/dl ($p = 0.049$). Occurrence of hypoglycaemia was similar in the 3 groups. Diabetes Quality of Life questionnaire (DQoL) showed improved patient satisfaction while SF-36 questionnaire pointed out better physical health scores in both CGM groups (respectively $p = 0.004$ and $p = 0.04$). The number of sensors used per month was significantly lower in G2 vs G1: $2.25 [1.27; 2.99]$ vs $3.42 [2.20; 3.91]$, $p < 0.001$. Improvement of A1c level was higher in patients on pump (G1+G2 vs G3: -0.7%) than on MDI (G1+G2 vs G3: -0.2%).

Conclusion: A long-term use of CGM resulted in a sustained improvement of glucose control and QoL in poorly-controlled T1D patients. A CGM prescription by the physician achieved a same improvement as a patient-led use, but with 34% fewer sensors.

Clinical Trial Registration Number: NCT00726440

Supported by: AFD

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Closed-loop glycaemic control over 36 hours in adolescents with type 1 diabetes

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Background and aims: We evaluated closed-loop (CL) insulin delivery during a 36-hour period replicating normal daily activities.

Materials and methods: Twelve adolescents with type 1 diabetes mellitus (T1D) (M 5; age 15.0±0.9 years; A1C 7.9±0.7%; BMI 21.4±2.6 kg/m²; duration of diabetes 6.1±2.8 years; total daily dose 1.0±0.4 U/kg/day; mean±SD) were studied at a clinical research facility on two occasions. Subjects were randomly allocated to receive either CL or open-loop (OL) (conventional CSII treatment) from 19:30 on day 1 for 36 h. During CL, basal rates on insulin pump were manually adjusted every 15 min as per advice of a model-predictive-control algorithm informed by real-time continuous glucose monitor. On each occasion, subjects engaged in normal daily activities (e.g. playing computer games, walks). They consumed meals (50–80 g CHO), accompanied by self-calculated insulin boluses, and snacks (15–30 g CHO). Moderate-intensity exercise on a stationary bicycle at 140 bpm heart-rate was performed at 10:40 (40 min) and at 17:30 (20 min).

Results: Overall mean plasma glucose levels were 7.2±1.2 mmol/l during CL versus 9.0±2.9 mmol/l during OL ($p=0.009$). Time spent in target glucose range 3.9–10 mmol/l was 82±10% vs 55±29% ($p=0.002$). Time above 10 mmol/l was 13±12% vs 37±33% ($p=0.005$) and time spent below 3.9 mmol/l was 5±4% vs 7±11% ($p=0.50$). Overnight, plasma glucose levels were in target for 97±7% vs 51±38% ($p=0.02$) and for 95±11% vs 45±45% ($p=0.001$) during the first and second night, respectively. Hypoglycaemia occurred on 11 occasions during OL vs 9 during CL (5 episodes were exercise-related, 4 occurred within 2 hours after meals).

Conclusion: Day-and-night CL may improve glucose control significantly compared to conventional CSII. Further adjustments are needed to optimise insulin delivery to minimise risk for hypoglycaemia after exercise and around meals.

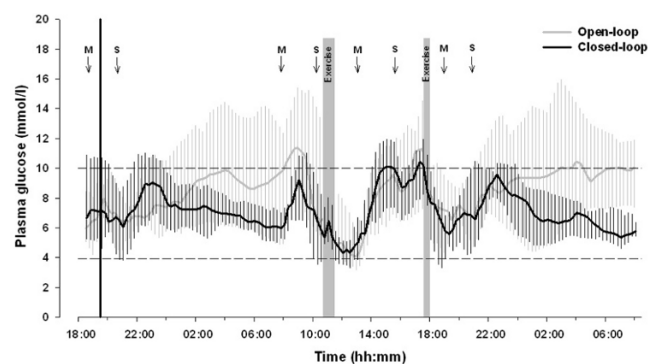


Figure. Plasma glucose levels (median, IQR) during closed-loop (black line) and open-loop (grey line) over the 36 h study period. Closed-loop started at 19:30 (vertical black line). Exercise sessions are shown by grey shadows. Vertical arrows illustrate when main meals (M) and snacks (S) were given.

Clinical Trial Registration Number: NCT01074801

Supported by: National Institute of Health

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Real time glucose monitoring system in patients with type 1 diabetes mellitus: systematic review and meta-analysis

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Background and aims: It's well known that tight metabolic control is crucial to prevent complications in type 1 diabetic (T1DM) patients. Conventional self blood glucose monitoring (SMBG) with glucose meter does not reflect the fluctuation of glucose level precisely enough. Continuous glucose monitoring system (CGMS) enables measurements in every 5 minutes, which provides more accurate data and can reduce the fear of hypoglycemia. Previous meta-analysis of randomized controlled trials (RCTs) using CGMS in a

retrospective way compared with SMBG did not show significant reduction in HbA1c in T1DM patients. Recently, several studies, many of them observational, have assessed the effect of real time CGMS (RT-CGMS) on metabolic control of type 1 diabetic patients. The results of the studies differed: a number of them have demonstrated a reduction in HbA1c with the RT-CGM, the other have not confirmed any benefits or noted that the benefit associated with continuous glucose monitoring was strongly related to age. Thus, the aim of this study was to explore the potential beneficial effects of the use of RT-CGM on diabetes management when compared with SBGM in patients with type 1 diabetes by conducting a systematic review and meta-analysis of randomized controlled trials.

Materials and methods: MEDLINE, EMBASE and The Cochrane Library were searched by two independent investigators for RCTs relevant to use RT-CGMS in patients with T1DM; additional references were obtained from the reviewed articles.

Results: 12 RCTs (n=1750 participants) met the inclusion criteria. Combined data from all studies showed better HbA1c reduction in subjects using RT-CGMS compared with SMBG (mean difference (MD) - 0.43; 95% CI: - 0.59 to - 0.27; $p<0.001$). The improvement in glycaemic control was observed in subjects treated with sensor-augmented insulin pump compared to both multiple daily injections or conventional insulin pump (8 RCTs, n=1075, MD - 0.51; 95% CI: - 0.76 to - 0.27; $p<0.001$). The beneficial effect of the real time CGM was observed in diabetic adults (n= 646, MD - 0.59; 95% CI: - 0.87 to - 0.31; $p<0.001$), as well as children (n= 464, MD - 0.34; 95% CI: - 0.52 to - 0.16; $p<0.001$). The reduction in HbA1c was noted in both groups with excellent or poor controlled type 1 diabetes. The benefits of applying the real-time CGM were not associated with increasing rate of acute hypoglycaemia.

Conclusion: Our meta-analysis confirmed that the use of the real time-CGMS compared with self-monitoring of blood glucose effectively lowers HbA1c in type 1 diabetes patients treated with continuous subcutaneous insulin infusion or multiple daily injections. The real-time CGMS is beneficial not only for people with poor diabetes control but also for well controlled diabetic patients. The use of this method improved glycaemic control both in adults and children, with type 1 diabetes.

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Successful transitions from MDI therapy to sensor-augmented pump therapy in the STAR 3 study: system settings and behaviours

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Background and aims: To successfully initiate sensor-augmented pump (SAP) therapy, appropriate initial settings for the insulin pump and the continuous glucose monitoring (CGM) subsystems are important. Subjects who realize rapid initial A1C reductions may employ different pump settings or adjustment strategies than subjects with minimal A1C reductions during the first 3 months after switching from MDI to SAP.

Materials and methods: The STAR 3 trial randomized 485 suboptimally-controlled subjects on multiple daily injection (MDI) therapy to either receive SAP therapy (n=244) or MDI therapy (n=241) for 1 year. At the end of the randomized phase, MDI subjects (n=204) entered the continuation phase of the study and switched to SAP therapy for 6 months and SAP subjects (n=216) continued on SAP. Subjects were grouped according to age, randomization assignment, and their A1C value after the first 3 months of SAP therapy (ie, month 3 for SAP subjects and month 15 for MDI subjects who switched). Pairwise comparisons between subjects in the highest and lowest A1C quartiles were made via t-test; significance of trends across all A1C values was evaluated via linear regression.

Results: Most subjects chose to continue with (89%) or switch to (85%) SAP therapy at 12 months. Subjects with the lowest A1C values 3 months after SAP initiation had lower baseline A1C values. These subjects consistently had lower glycemic variability (SD of CGM values) than subjects with higher A1C values. Subjects with the highest A1C values averaged fewer daily boluses, used the Bolus Wizard calculator less often, and wore CGM sensors less frequently than subjects with the lowest A1C values after 3 months on the pump. These results held for subjects who began SAP therapy at the beginning of the study and for those who switched to SAP therapy after 12 months in the MDI arm of the study. Among the 204 MDI subjects who switched, those with the lowest A1C values after 3 months of SAP therapy had adjusted their basal in-

sulin delivery rates more than twice as often as subjects with the highest A1C values. The trends and pairwise differences were not statistically significant across all 4 patient subgroups (Table).

Conclusion: Successful transitions of patients from MDI to SAP therapy, as assessed by A1C values after 3 months of SAP use, are associated with frequent use of CGM sensors, frequent bolus dosing, smaller bolus doses, and greater use of the Bolus Wizard calculator compared to patients who realize only modest A1C improvements in the initial 3 months of pump therapy. For subjects who have attempted optimal management with MDI, favorable initial decreases in A1C with SAP therapy may be associated with frequent changes to the pump's basal insulin delivery rate.

Comparison of subjects after 3 months SAP therapy.

*, $P < 0.05$ (pairwise); ‡, $P < 0.05$ (overall trend)

Randomized to SAP (n=244)	Lowest A1C quartile, age ≥ 19 (6.48%, n=49)	Highest A1C quartile, age ≥ 19 (8.21%, n=41)	Lowest A1C quartile, age < 19 (6.65%, n=21)	Highest A1C quartile, age < 19 (8.57%, n=14)
Baseline A1C (%)	8.05	8.62 [‡]	7.98	8.72 [‡]
SD of CGM values (mg/dl)	52.4	65.9 [‡]	57.5	77.4 [‡]
Sensor wear (% of time)	66%	61% [‡]	63%	56%
Boluses/day (Bolus Wizard uses/day)	5.2 (4.8)	5.0 (4.3)	6.0 (5.6)	5.2 [‡] (4.7 [‡])
Adjustments to basal rate profile (n)	12.9	14.2	11.5	11.4
Switched to SAP (n=204)	Lowest A1C quartile, age ≥ 19 (6.65%, n=41)	Highest A1C quartile, age ≥ 19 (8.42%, n=35)	Lowest A1C quartile, age < 19 (6.93%, n=17)	Highest A1C quartile, age < 19 (9.00%, n=14)
Baseline A1C (%)	7.29	8.55 [‡]	7.79	8.79 [‡]
SD of CGM values (mg/dl)	47.3	62.2 [‡]	61.9	73.7 [‡]
Sensor wear (% of time)	58%	45% [‡]	55%	43%
Boluses/day (Bolus Wizard uses/day)	5.1 (4.3)	4.5 (4.0)	5.8 (5.8)	5.1 (4.7)
Adjustments to basal rate profile (n)	15.9	7.6	12.9	4.7 [‡]

Clinical Trial Registration Number: NCT00417989

Supported by: Medtronic, Inc.

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Influence of time-point of calibration on accuracy of a continuous glucose monitoring system

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Background and aims: Currently used continuous glucose monitoring systems (CGMS) need to be calibrated by self measured blood glucose (SMBG) values. Data on the influence of the time-points of calibration on accuracy of CGMS is scarce. In general, calibrations are recommended during stable glycaemia and, therefore, essentially in fasting or pre-prandial situations. However, in clinical practice the possibility of less strict calibration modes (including post-prandial SMBG) may improve adherence of patients. The aim of the present study was to investigate whether the pattern of calibration (predominantly pre-prandial vs. predominantly post-prandial) has an influence on sensor accuracy and whether this effect differs according to the glycaemic level.

Material and methods: Twenty individuals with type 1 diabetes (mean \pm SEM age 35.9 \pm 2.9 years, HbA_{1c} 7.3 \pm 0.3 %, diabetes duration 17.2 \pm 2.1 years) were included into the study. Two CGMS Guardian-RT[®] (Medtronic, Minneapolis, USA) were applied simultaneously with sensors inserted into the lower abdomen on either side. One sensor was calibrated predominantly using pre-prandial SMBG (Calibration_{PRE}: two preprandial values and one bedtime value). The other sensor was calibrated predominantly using post-prandial SMBG (Calibration_{POST}: two postprandial values and one fasting value). The bedtime value in Calibration_{PRE} and the fasting value in Calibration_{POST} had to be included due to technical requirements of the CGMS system. A minimum of 3 additional SMBG per day were obtained for analysis of accuracy. Sensor readings of each calibration pattern were divided into 4 categories according to the glycaemic range of the reference values (low \leq 4 mmol/l; euglycaemic 4.1–7 mmol/l; hyperglycaemic I 7.1–14 mmol/l and hyperglycaemic II $>$ 14 mmol/l).

Results: The overall mean \pm SEM absolute relative difference (MARD) between capillary reference values and sensor readings was 18.3 \pm 0.8% for Calibration_{PRE} and 21.9 \pm 1.2% for Calibration_{POST}, respectively ($p < 0.001$). MARD according to glycaemic range was 47.4 \pm 6.5% (low), 17.4 \pm 1.3% (euglycaemic), 15.0 \pm 0.8% (hyperglycaemic I), and 17.7 \pm 1.9% (hyperglycaemic II) for Calibration_{PRE} and 67.5 \pm 9.5% (low), 24.2 \pm 1.8% (euglycaemic), 15.5 \pm 0.9% (hyperglycaemic I), and 15.3 \pm 1.9% (hyperglycaemic II) for Calibration_{POST}, respectively ($p < 0.001$ for difference within subgroups for both calibration patterns). In low and euglycaemic ranges MARD was significantly lower in

Calibration_{PRE} compared with Calibration_{POST} ($p = 0.007$ and $p < 0.001$, respectively).

Conclusions: Sensor calibration predominantly based on pre-prandial SMBG resulted in a significantly higher overall sensor accuracy compared with a predominantly post-prandial calibration. The difference is most pronounced in the hypo- and euglycaemic reference range whereas both calibration patterns were comparable in the hyperglycaemic range.

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Prognosis of diabetes related complications by continuous glucose monitoring profiles: data of the JDRF study analysed by the glucose-pentagon-model

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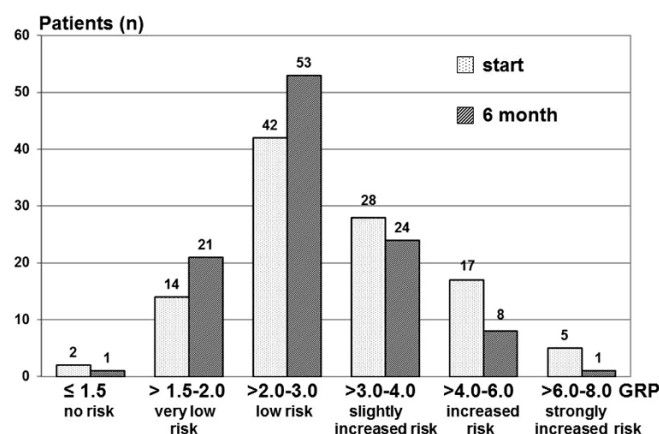
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Background and aims: Variability in glycaemia in patients with diabetes can be documented by means of continuous glucose monitoring (CGM) but not with capillary self-monitoring of blood glucose or HbA1c measurement. The glucose pentagon model (GPM) takes four parameters obtained by analysis of CGM recordings beside the HbA1c into account. By doing so a risk parameter (GRP) derived from the GPM might allow a better prognosis of the risk to develop diabetes related complications (DRCs) than the HbA1c by taking variability parameters into account.

Materials and methods: Data from 108 patients from the JDRF study (Medtronic CGM-System only) were analyzed: CGM profiles from the start of the study and after 6-month were analysed by a software that generates the GPM and calculates the GRP. The change in this risk parameter was compared to the risk indicated by the change in HbA1c.

Results: The improvement in HbA1c from 7.4% to 7.0% during this study was accompanied by a reduction in mean glucose (from 163 to 156 mg/dL), standard deviation (61 to 57 mg/dL), AUC $>$ 160 mg/dL (29.2 to 23.1), and time per day $>$ 160 mg/dL (634 to 576 min). The reduction in a risk parameter calculated from the GPM from 3.3 to 2.7 by 18.2% was larger than the risk reduction by 8.6% indicated by the HbA1c.

Conclusion: In summary, the prognosis for the development of DRCs by using the GPM appears to be more valid than that provided by the HbA1c. Long-term studies will more definitively proof that parameters describing variability in glycemia should be part of such risk parameters. (We would like to thank the JDRF for providing us the data for this analysis.)



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Glucose sensor performance during pressure changes

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Background and aims: There is a need to provide people with diabetes a reliable device that continuously measures glucose in hypo- and hyperbaric conditions as during SCUBA diving when 30 meter depth corresponding to

4 atm and in commercial aircrafts with a cabin pressure of 0.75 atm. The aim of this study was to test Medtronic's new Enlite glucose sensor in hypobaric and hyperbaric environments, providing evidence that sensor performance is acceptable.

Materials and methods: This feasibility study was conducted in a pressure chamber. A non-diabetic subject was chosen to be studied allowing for non-glucose specific changes in signal to be evaluated. Half of the sensors were pre-wetted with sterile saline prior to subcutaneous insertion. On Day 1 of the study, the subject had 24 Enlite glucose sensors inserted (right side of the body, 12 abdominal and 12 lower back), with the lower back sensors being pre-wetted. After the 2 h sensor initialization period and a stabilization period at 1.0 atm, the chamber pressure was ramped down to 0.5 atm (~10 kPa O₂) for a period of 20 min, raised to 0.75 atm (~16 kPa O₂), and then returned to 1.0 atm (21 kPa O₂). During this time, frequent blood glucose samples were taken via Hemocue blood glucose monitor every five minutes. On Day 2 of the study, the subject was again inserted with 24 Enlite glucose sensors (left side of the body, 12 abdominal and 12 lower back), with the abdominal sensors being pre-wetted prior to insertion. After 2 h initialization, the chamber pressure was increased to 4.0 atm (~84 kPa O₂) for a period of 20 min, followed by a decompression to 1.0 atm. During the exposure to varying pressures, a parallel in-vitro test was run using eight Enlite sensors connected to a 1-min Medtronic iPro and eight Enlite sensors connected to a 5-min MiniLink linked to a Guardian receiver.

Results: The in-vivo/vitro signals and blood glucose values were processed to mimic what a user of a Medtronic CGM device would see in real-time. On Day 1, a total of 266 paired sensor/meter points were collected in-vitro yielding an overall MARD of 14.9%, with 100% being in the Clarke A+B region. Although the analysis of the sensor sensitivity showed a slight decrease ($p < 0.05$) during hypobaric conditions, the change was well within the sensitivity thresholds of 1.5–15 as defined by the real-time device. On Day 2, a total of 339 paired sensor/meter points were collected, yielding an overall MARD of 6.69%, with 100% being in the Clarke A+B region. Analysis of the sensor sensitivity showed no significant change in sensor sensitivity ($p = 0.20$). Results from the in-vitro test under the same conditions demonstrated linearity (R^2) of 0.98. There was no significant change in sensitivity during hyperbaric conditions ($p = 0.18$) but there was a change during hypobaric conditions ($p < 0.05$), however the sensitivity was well within the sensitivity thresholds of 1.5–15 as defined by the real-time device.

Conclusion: The results from this feasibility study demonstrate that use of the Medtronic Enlite sensor under hypobaric and hyperbaric conditions showed robust sensor and system performance. Further studies on type 1 diabetic subjects are needed under the same pressure conditions.

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INTERPRET, an international report on routine practice of sensor-enabled pump therapy: result from the 6 months interim analysis

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Background and aims: Randomized controlled trials (RCT) have shown significant reduction in HbA1c with use of continuous glucose monitoring (CGM) combined to insulin pump in type 1 diabetes (T1DM) treatment. In these RCT, sensor usage frequency was shown to impact on the clinical outcomes. The aim of this study is to document the international routine practice in sensor usage in T1DM patients treated with sensor-augmented pump (SAP) therapy. We assess if variables such as frequency of sensor usage, age, HbA1c, and main indication to start CGM are associated with an improvement in clinical outcomes and observe which individuals are benefiting the most from the SAP therapy.

Materials and methods: This international multi-center prospective observational study was conducted in adults and children with T1DM treated by insulin pump therapy for more than 6 months at the time of enrolment and whose physicians decided to treat with SAP. Twenty five clinics from 15 countries participated to the data collection. Patients were followed up for 1 year

and clinical outcomes were assessed every 3 months. An interim analysis is performed on the 6 months follow-up data.

Results: 263 T1DM patients (mean age: 28.0 ± 15.7 years [range 1–69]; BMI: 23.3 ± 4.9 kg/m²; diabetes duration: 13.9 ± 10.7 years and 38% male and 62% female) were eligible for data analyses. Mean HbA1c at baseline was $8.1 \pm 1.4\%$ and 82% of the patients had suboptimal HbA1c $> 7\%$. After 3 months of treatment, the HbA1c change was significantly correlated to sensor usage (Spearman correlation coefficient 0.128; $p = 0.046$) in the overall population. Factors associated with improvement in HbA1c after 6 months of treatment were high HbA1c levels at baseline ($p < 0.0001$), more frequent sensor use ($p = 0.021$) and age group ($p = 0.016$). As shown in the figure 1, the use of sensors was higher in young children (0–7y) and adults ($> 24y$), and the improvement in HbA1c in these groups from baseline was better than in the other age groups, 0.37% and 0.27%, respectively.

Conclusion: This is the first, large observational study providing data from real life practice that shows that initial baseline HbA1c levels, sensor usage compliance and age are significantly associated with reduction of HbA1c levels after 6 months of treatment. Longer-term data will provide further insights as to the clinical practices and outcomes of CGM treatment in real-life.

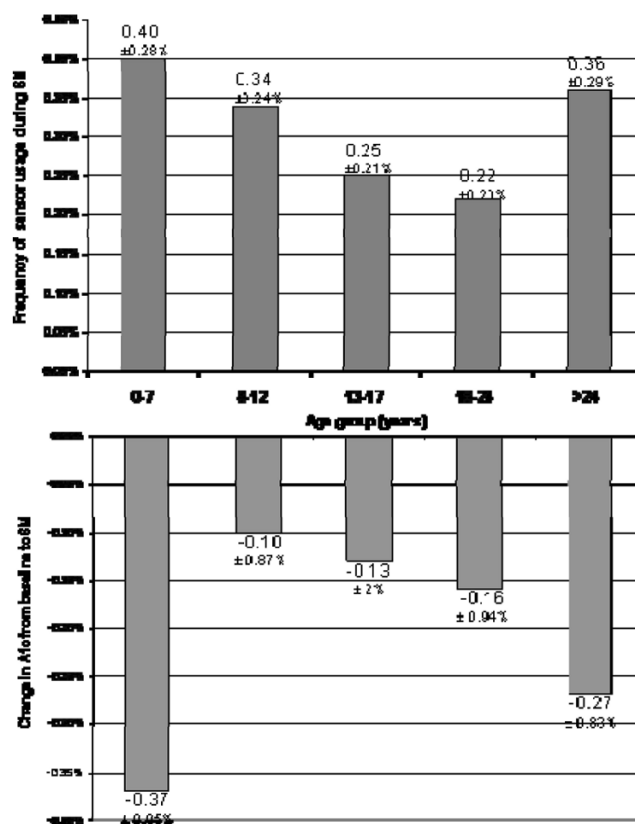


Figure 1: Change in HbA1c at 6M and sensor usage frequency (reported as mean ± SD) by age group

Clinical Trial Registration Number: NCT00790088
Supported by: Medtronic

PS 085 Insulin pumps: treatment

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Long-term continuous subcutaneous insulin infusion systems in daily clinical practice: effectiveness and safety

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Background and aims: The aims of this study are: 1) to examine the clinical effectiveness of continuous subcutaneous insulin infusion (CSII) systems over a 5-year follow-up period in type I diabetic patients. We will consider the overall clinical situation and the main cause for the patient's indication. 2) assess CIIS safety.

Materials and methods: We performed a descriptive, observational study including all CSII patients until January 2011. We analysed the glycated haemoglobin controls (HbA_{1c}) at the start of therapy and post-CSII at: 1 month, 3 months, 6 months, and every year for 5 years. We examined the following CSII-related complications: hyperglycaemic crises (ketotic, non-ketotic and ketoacidotic [KAD]), local problems (abscesses and lipodystrophies) and severe hypoglycaemic crises.

Results: We attended to 149 type 1 diabetic patients (35 men and 114 women) treated with CSII over an average of 3.59 ± 2.92 years. CSII was implanted for the following reasons: 49 (33%) gestational diabetes, 43 (27%) poor chronic glycaemic control, 40 (29%) glycaemic instability or unstable diabetes, 6 (4%) unnoticed hypoglycaemia, 2 (1%), dawn phenomenon, and 9 (6%) were indicated for several causes. Initial HbA_{1c} was $8.40 \pm 1.28\%$, which decreased to a minimum of $7.17 \pm 1.04\%$ three months after therapy was started ($P < .05$) and remained low at four years follow-up ($7.51 \pm 0.99\%$ [$P < .05$ versus baseline HbA_{1c}]). CSII subgroups: the group with poor metabolic control had a baseline HbA_{1c} of $8.99 \pm 1.29\%$ which decreased after 3 months to $7.39 \pm 1.35\%$ ($P < .05$). This decrease was maintained over 2 years ($7.34 \pm 0.94\%$; $P < .05$ versus baseline HbA_{1c}). The unstable glycaemic group's pre-implantation HbA_{1c} was $8.16 \pm 0.92\%$, which decreased to $7.18 \pm 0.73\%$ after 3 months ($P < .05$) and $7.34 \pm 0.89\%$ at 6 months ($P < .05$ versus baseline HbA_{1c}). We observed 6 severe local problems at the catheter insertion site (2 abscesses that required drainage and 3 partial lipodystrophies) and 32 hyperglycaemic crises, 16 of which were KAD, resulting in a KAD incidence of 0.039 episodes/patient/year. The incidence of severe hypoglycaemic crises was 0.017/patient/year.

Conclusion: 1) CSII therapy improved glycaemic control for the first 4 years of follow-up. We have observed differences in the reduction of HbA_{1c} in the group with glycaemic instability or unstable diabetes at 6 months, and in the poor control group at 2 years. 2) CSII treatment was safe. Catheter-related local complications have been infrequent and the incidence of KAD and hypoglycaemic crises is similar to those reported in other studies.

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Insulin pump therapy may be equally effective in elderly and young type 1 diabetes patients

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Background and aims: It is generally accepted that in type 1 diabetes (T1DM) continuous subcutaneous insulin infusion (CSII) via a personal pump is more effective than the multiple daily injections (MDI) model. It is not clear, however, whether all age groups of adult T1DM patients may equally benefit from CSII therapy. Especially, the effectiveness of this new technology-based therapy in elderly patients may be of concern.

Materials and methods: We aimed to compare the glycaemic control and use of selected pump tools in T1DM patients on CSII over the age of 50 (50+ T1DM) with younger subjects. The last available insulin pump/blood glucose meter downloads and last available HbA_{1c} level of 102 adult T1DM subjects on CSII treatment were reviewed. We have divided our population into 2 subgroups: 50+T1DM patients ($n=10$, mean age: 57.3 ± 7.16 years, duration of diabetes: 24.11 ± 8.05 years, duration on CSII: 6.01 ± 4.19 years) and younger individuals: ($n=92$, age: 26.39 ± 7.71 years, duration of diabetes: 12.41 ± 7.1 years, duration on CSII 3.93 ± 2.75 years).

Results: There were no differences in glycaemic control achieved with CSII treatment by 50+ T1DM patients vs. younger subjects: the HbA_{1c} levels were $6.98 \pm 1.04\%$ and $7.13 \pm 1.16\%$ ($p=0.67$), the mean glycemia based on glucometer downloads was 139 ± 29 mg/dL and 142 ± 36 mg/dL ($p=0.55$), respectively. Interestingly enough, there were no differences with respect to the use of important personal pump options and tools such as daily number of boluses, basal/bolus ratio, frequency of usage of dual-wave/square bolus function and bolus calculator option, percentage of patients using continuous glucose monitoring. 50+ T1DM individuals required more insulin per kilogram (0.71 j/kg vs. 0.56 j/kg for older and younger individuals, respectively, $p=0.036$).

Conclusion: In conclusion, insulin pump therapy can be equally effective in T1DM patients older than 50 and in younger adult subjects with this disease.

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Quality of life and subcutaneous ambulatory insulin pump in type 2 diabetes patients

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Background and aims: Does an insulin pump therapy in diabetic patients provide, beside a better metabolic control, an increase in quality of life, while remaining easy to use?

Materials and methods: 189 Type 2 diabetes patients (age: 58.5 ± 10.2 yrs, sex ratio M/F: 0.4, diagnosed for diabetes for 17.5 ± 9.1 years, weight: 93.3 ± 19.3 kg) switched to an ambulatory subcutaneous insulin pump therapy (glucose control failure in despite of 3.22 ± 1.46 insulin injections/day) are analyzed in an observational study. 139 patients went on with their insulin pump; 8.3% gave up with the pump and among the 139 patients followed after 1 year (T1Y), 90 (65%) have agreed to answer and filled in a Quality of Life questionnaire.

Results: The pumps that were used are of the following types: Accu check spirit (9%), Animas (8%), Cozmo (18%) and Medtronic (65%), and the most used Catheter was ($n=59-51\%$) the QUICK SET type. The analysis on the patient's feeling towards the pump can be classified in: poor - medium - good and concerns: the estimate of general state, the limitation in physical activity and everyday life and the moral state. At T0, only 3.3% of the patients view their general state as good, 67.8% as medium and 28.9% as poor. The physical activity status and everyday life is considered as good by 29.4% of the patients, medium by 44.6% and poor by 26.1%. Regarding the moral status, 31.5% consider it as good, 60.9% medium and 7.6% poor.

Conclusion: The metabolic optimisation through insulin s /c pump after poor glucose corol in despite multi insulin injections treatment, lead, in a large majority of type 2 diabetes patients, to a great improvement of quality of life, with better physical and moral health. Almost all considers the use of the pump is simple. They are satisfied to a better experience and better control thanks to the latter.

Health status and pump (after 1 year)			
	Easy	~ Easy	Difficult
Pump therapy after 1 year, the analysis of the feeling towards the pump: learning and usage ease	58.6%	36.2%	5.2%
Evolutionary feeling of the health status	76.4%	16.4%	7.3%
	Yes	Better	Bad
Efficacy provided by the pump on glycaemic status	45.5%	52.7%	1.8%
	Very good	~ Good	Bad
Feeling and usage towards the pump	74.1%	19.0%	6.9%

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Continuous subcutaneous insulin infusion is relevant in reducing insulin requirement in patients with type 1 diabetesE. Pankowska¹, M. Błazik¹, L. Groele²;¹Pediatrics, Institut of Mother and Child, ²Pediatrics, Medical University of Warsaw, Poland.

Background and aim: Continuous subcutaneous insulin infusion (CSII) is an alternative to multiply daily injection (MDI) method of insulin delivering in type 1 diabetes patients. In numerous studies has been proofed that CSII reduced the risk of sever hypoglycemia, improve of metabolic control and quality of life in short and longer time of observations. We are still lack of evidences on the efficiency of this method in the term of individual insulin requirement. The aim was to assess the insulin requirement in total (TDDir) and basal daily dose (BDDir) in CSII comparing to MDI method of insulin delivering.

Material and method: The parallel, day-to-day study compared MDI to CSII enrolled 41 pediatrics patients in age 1,3 to 17,9 ys and diabetes duration from 0,01 to 12,7ys and median HbA1c 7,3 from 6,0 to 12,8%. 15 patients in MDI used long action analogues (LAA) and 23 NPH insulin (SAA), 3 patients left without basal insulin; 31 short acting analogues and 10 regular insulin.

Results: The insulin requirement was reduced by 28% in basal and total daily insulin dose. Analysis by the age of patients showed the highest reduction in TDDir by 37% ($p<0,005$) in the youngest group of patients aged 0-7ys. The significant reduction of BDDir by 45% was in children aged 7-12 and by 31% in children above 12 ys. Moreover, lower but not significantly glycemia profile (med. 120,5 mg/dl vs 130,5 mg/dl; $p=0,4$) occurred in CSII method. Considering the type of basal insulin in MDI method reduction by 15% ($p=0,51$) was in LAA group and by 47% in NPH group ($p=0,002$).

Conclusions: CSII method has an impact on reduction in total and basal daily insulin requirement. In the algorithm of introducing CSII method, the reduction of TDD -MDI by 30% should be considered, particularly in pre-schoolers and in patients used NPH insulin.

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Measures of oxidative stress and anti-oxidant capacity in plasma samples from patients with type 2 diabetes before and after insulin pump therapyS.J. Setford¹, H. Anhalt¹, I. Megson³, J.P. Frias², A. Treweek³;¹Research & Development, LifeScan Scotland Ltd, Inverness, UK, ²Animas Corporation, West Chester, USA, ³UHI Millenium Institute, Inverness, UK.

Background and aims: A cohort of 56 poorly controlled (A1C $8.4\pm1.3\%$) insulin pump-naïve patients consisting of three groups (two or more oral anti-diabetes agents [OADs]; basal insulin with or without OADs; basal-bolus insulin with or without OADs) was subject to a 16 week multi-centre pilot study of insulin pump therapy.

Materials and methods: All diabetes medications except for metformin were discontinued on initiation of pump therapy. Most patients were managed with 1 or 2 basal rates with resultant mean A1C improvement of $1.2\pm1.2\%$ ($P<0.001$). A number of serum biomarker species were determined at initiation and termination of the study including: A1C; insulin; pro-insulin; a measure of antioxidant capacity (oxygen radical antioxidant capacity, ORAC); and measures of oxidative stress (oxidised low density lipoprotein, ox-LDL; protein carbonyls; malondialdehyde, MDA)

Results: Consistent with our hypothesis that improvement in glycemic control using insulin pump therapy in these subjects would result in decrease of these markers, levels of insulin, pro-insulin and oxidative stress markers were depressed by ~10-35% (Table), as compared to baseline. A ~4% depression in ORAC was also observed, a measure that would be expected to increase in the face of reduced oxidative stress. Whilst samples were collected in the fasting state the role of dietary influences cannot be ruled out.

Conclusion: This 16 week pilot study using insulin pump therapy in poorly controlled patients with type 2 diabetes demonstrated markers of oxidative stress and anti-oxidant capacity to decrease, potentially decreasing mediators of inflammation and atherogenesis, in individuals with type 2 diabetes. Future studies should consider measures of endothelial function, coupled with a mechanistic approach to establish the relationship between insulin pump therapy and oxidative stress and to determine if the reduction in glycemia, as indicated by A1C reduction, is the main driver of the observed decrease in oxidative stress markers.

Marker	Change (%)	P
Insulin (pM)	-22.7	0.036
Proinsulin (pM)	-35.4	0.024
MDA (μ M)	-19.4	<0.0001
ORAC (μ IU/mL)	-3.9	0.013
Protein carbonyls (nmol/L)	-15.1	<0.0001
Ox-LDL (U/I)	-10.5	0.033

Supported by: Animas Corporation

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Long term follow-up in children and adolescents with type 1 diabetes using insulin pump therapy: a retrospective multicentre, multinational study (Input(log) Study)C. Mameli¹, A.E. Scaramuzza¹, J. Ho², R. Cardona-Hernandez³, L. Suarez-Ortega³, G. Zuccotti¹;¹Paediatrics, University of Milano - Luigi Sacco Hospital, Italy, ²Paediatrics, Faculty of Medicine, University of Calgary, Calgary, Canada, ³Endocrinology and Diabetes, Sant Joan de Déu Hospital, Barcellona, Spain.

Background and aims: Few data are available regarding long term follow up in children and adolescents with type 1 diabetes (T1DM) using continuous subcutaneous insulin infusion (CSII). The aim of this study was to evaluate the long-term glycaemic control in children and adolescents with T1DM, using CSII for at least 4 years in three Diabetes Centres from 3 different countries: Italy, Canada and Spain.

Materials and methods: Each centre participating in the study reviewed charts of patients with T1DM, using CSII. Inclusion criteria to enrol patients were: age 4 to 20 yrs., T1DM for more than 4 yrs. and CSII therapy for at least 4 yrs. Data collected included gender, age, T1DM duration, date of CSII initiation, body mass index (BMI), HbA1c, insulin requirement at baseline and every 6 months, and DKA and severe hypoglycaemic episodes at baseline and during follow-up.

Results: After reviewing the charts, 126 patients fulfilling the inclusion criteria were found. Twenty-five of them were excluded because of data missing. We present the data of 101 patients (61 males), aged 4-20 yrs. (mean: 14.6 ± 3.8 yrs.), with T1DM from 9.7 ± 3.3 yrs., using CSII for 5.6 ± 1.7 yrs. (range 4-11 yrs.). HbA1c data are shown in the table. After CSII initiation HbA1c showed a significant improvement only in the first year. During the follow-up, HbA1c values tended to increase. Evaluating HbA1c according to Countries, a difference has been observed with slightly lower values in Italy than in Canada and Spain. When evaluated as a whole group or by Countries, no significant differences were observed for BMI, insulin requirement, severe hypoglycaemia and DKA episodes from baseline throughout the follow-up.

Conclusion: CSII therapy seems an effective therapy in the long term, although the major benefit in HbA1c is seen in the first year of CSII initiation. In this preliminary study, a difference in HbA1c during long term follow-up was observed among Countries. Further studies will evaluate which factors may be responsible for this observation (i.e., different dietetic habits, lifestyle, etc.). Table - HbA1c values according to Country and as a whole. T=time; significance among groups has been evaluated by Kruskal Wallis test: * $p=0.03$; * $p=0.003$; when evaluated as a whole HbA1c has been evaluated using paired test statistic vs. baseline: § $p=0.005$; ° $p=0.005$

	Baseline	T +6mo	T +12mo	T +24mo	T +36mo	T +48mo	T +60mo	Last visit
Canada	$8.17\pm1.02^*$	7.78 ± 0.91	8.09 ± 1.24	$8.23\pm1.18^*$	$8.30\pm1.07^*$	$8.40\pm1.05^*$	8.16 ± 0.96	$8.51\pm1.22^*$
Italy	$8.08\pm0.99^*$	7.73 ± 0.78	7.69 ± 0.96	$7.51\pm0.79^*$	$7.67\pm0.82^*$	$7.61\pm0.78^*$	7.81 ± 0.33	$7.83\pm0.72^*$
Spain	$9.08\pm1.36^*$	7.95 ± 0.78	8.14 ± 0.51	$8.61\pm0.92^*$	$8.12\pm0.53^*$	$8.45\pm0.75^*$	8.18 ± 0.63	$8.51\pm0.64^*$
All	8.52 ± 1.25	$7.83\pm0.81§$	$7.95\pm0.85§$	8.10 ± 1.18	$8.07\pm1.02§$	8.24 ± 1.07	$8.27\pm1.09^*$	8.33 ± 1.04

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The low glucose suspend function in sensor-augmented pump therapy prevents hypoglycaemia in children

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Background and aims: The sensor-augmented insulin pump (SAP) "Paradigm VEO" system offers a novel automatic insulin shut-off mechanism low glucose suspend (LGS) possibly preventing severe hypoglycaemia. In a prospective study, we investigated the effect of the LGS algorithm on the frequency of hypoglycemia in children and adolescents with type 1 diabetes under real-life conditions.

Materials and methods: Twenty-one patients with type 1 diabetes (age 10.8 ± 3.8 years, diabetes duration 5.9 ± 3.0 years, pump therapy 3.7 ± 1.7 years, A1C $7.8 \pm 1.1\%$) from 3 pediatric centers used the Paradigm VEO system (PR-VEO) during two subsequent time periods: SAP without LGS for two weeks and then SAP with LGS enabled for 6 weeks. The primary objective was to assess the frequency of hypoglycemic episodes when using the LGS feature with an insulin delivery shut-off of max 2 hours at a sensor glucose level below 70 mg/dL (3.9 mmol/L).

Results: A total of 1298 LGS alerts occurred, 853 were shorter than 5 minutes as patients reacted immediately and no interruption of insulin delivery took place. The frequency of LGS alerts was 2.56 ± 1.86 per patient/day (6am–10pm: 76%). Of all LGS episodes, 42% lasted less than 30 min while 24% took more than 120 min, respectively. LGS >120 min was more frequent in the night (84%). The AUC <70 mg/dL was decreased by using LGS (SAP vs. SAP+LGS: $0.76 \text{ mg/dL} \times \text{day}$ vs. $0.53 \text{ mg/dL} \times \text{day}$, $p=0.05$) as well as the time spent in hypoglycemia (average minutes/day: $101 \pm 68 \text{ min}$ vs. $58 \pm 33 \text{ min}$, $p=0.002$). Also, the number of hypoglycemic excursions was significantly reduced during SAP+LGS (excursions <70mg/day 1.27 ± 0.75 vs. 0.95 ± 0.49 , $p=0.01$, excursions $\leq 40 \text{ mg/dL}$: 0.28 ± 0.18 vs. $0.13 \pm 0.14 \text{ mg/dL/day}$, $p=0.005$) with no difference in the mean glucose level (145 ± 23 vs. $148 \pm 19 \text{ mg/dL}$). Regarding safety, no episodes of severe hyperglycemias or DKA were observed following LGS.

Conclusion: The present study provides evidence for reducing the risk for hypoglycemia with LGS without compromising the safety of CSII therapy.

Supported by: Medtronic

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Continuous subcutaneous insulin infusion (CSII) in type 2 diabetes: reductions in HbA_{1c} - mean glucose and variability

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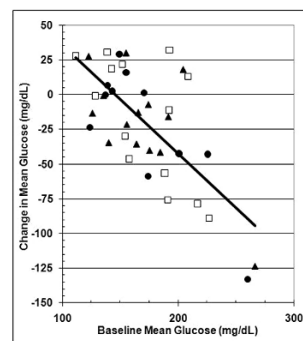
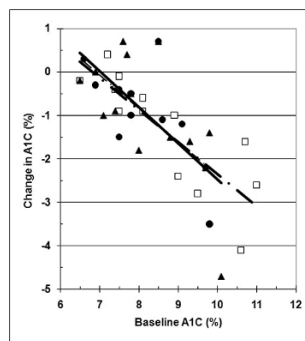
¹Animas Corporation, West Chester, ²Biomedical Informatics Consultants LLC, Clinical Research and Biostatistics, Potomac, ³AMCR Institute Inc, Escondido, ⁴Atlanta Diabetes Associates, Atlanta, ⁵B2S Consulting, Carmel, ⁶UCSD/VA TCOYD, San Diego, ⁷Cetero Research/Diabetes and Glandular Disease Clinic, San Antonio, USA.

Background and aims: Insulin infusion therapy has become a viable alternative for insulin deficient patients with type 2 diabetes. This study was designed to study clinical outcomes in poorly controlled patients with type 2 diabetes after initiating CSII.

Materials and methods: We evaluated the effect of CSII therapy in a 16 week open-label pilot study. 56 insulin pump naïve patients with type 2 diabetes previously treated with A) 2 oral agents alone B) oral agents plus basal insulin, or C) oral agents plus Multiple Daily Injection (MDI) insulin therapy were switched to CSII and monitored with Continuous Glucose Monitoring (CGM) at baseline, 1, 2, 3, 4, and 16 weeks.

Results: There were significant reductions in HbA_{1c}, mean glucose, SD of glucose within days, hyperglycemia, and percentiles of the glucose distributions. Reductions of HbA_{1c} and mean glucose were linearly related to baseline HbA_{1c} with similar relationships irrespective of previous treatment (Fig.). Most improvement occurred in the initial 4 weeks. Subjects with baseline A1C 9%-10% showed the most dramatic response. There was a small increase in risk of non-severe hypoglycemia.

Conclusion: Use of CSII rapidly improves glycemic control in patients with T2DM as documented by CGM; these effects are more significant after adjustment for baseline HbA_{1c} or mean glucose.



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Improving the estimation of mealtime insulin dose in adults with type 1 diabetes: Normal Insulin Demand for Dose Adjustment (NIDDA study)

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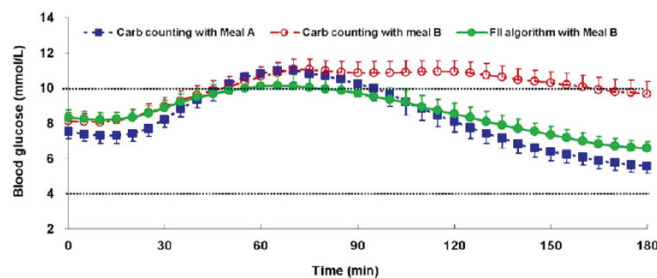
Macquarie University, Sydney, Australia.

Background and aims: Although carbohydrate counting is routine practice in type 1 diabetes, hyperglycaemic episodes are common. A food insulin index (FII) has been developed and validated for predicting the normal insulin demand generated by mixed meals in healthy adults. We sought to compare a novel algorithm based on the FII for estimating mealtime insulin dose with carbohydrate counting in adults with type 1 diabetes.

Materials and methods: Twenty eight patients using insulin-pump therapy consumed two different breakfast meals of equal energy, glycemic index, fibre and calculated insulin demand (both FII = 60) but ~2-fold difference in carbohydrate content, in random order on 3 consecutive mornings. On one occasion, carbohydrate counting algorithm was applied to meal A (75 g carbohydrate) for determining bolus insulin dose. On the other two occasions, carbohydrate counting (about half the insulin dose as meal A) and the FII algorithm (same dose as meal A) were applied to meal B (41 g carbohydrate). Real-time continuous glucose monitor (CGMS) was used to assess 3-h postprandial glycemia.

Results: Compared with carbohydrate counting, the FII algorithm significantly decreased glucose incremental area under the curve over 3h (-52%, $P < 0.01$), peak glucose excursion (-41%, $P = 0.001$) and improved the percentage of time within the normal BGL range (4–10 mmol/L) (+31%, $P < 0.001$). There was no significant difference in the occurrence of hypoglycemia.

Conclusion: The findings support the use of a FII algorithm based on insulin demand in healthy subjects to optimize glycemic control without increasing the risk of hypoglycemia in patients with type 1 diabetes.



Clinical Trial Registration Number: ACTRN12609001034224

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The impact of the bolus and food calculator „Diabetics“ on decreasing postprandial glucose variability in children with type 1 diabetes treated with insulin pump: the results of RCT

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Background and aims: Fluctuations of blood glucose levels expressed by the glucose variability is one of the main factors frustrating patients with diabetes in their daily self-management. Moreover, it is often masked by targeted level of HbA_{1c}. Bolus calculators as digital tools have to facilitate the process of insulin programming and are increasingly being introduced in treatment of patients with type 1 diabetes. The aim of the study was to determine whether the digital bolus calculator compared to traditional insulin programming has an impact on postprandial and diurnal glucose variability in children with type 1 diabetes treated with insulin pumps.

Material and methods: This 3-month, randomized, open-label study included 48 children aged 1–18 years. All patients were trained in food counting where carbohydrate unit (CU) and fat-protein unit (FPU) were taken into account in prandial insulin dosing by normal-wave or dual-wave boluses. Patients were subsequently randomly allocated to the experimental group (GA) which used digital bolus calculator ‘Diabetics’ for food and bolus calculation and to group B (GB) which used caloric tables. The parameters of glucose variability were estimated based on 8-point glucose profile (mg/dl).

Results: We observed significant differences between groups in all postprandial glucose variability parameters described by SD as well as the mean of all glucose values (Mean_T), SD of all glucose values (SD_T), SD of all glucose values within days (SD_W), SD between time points in 8-point glucose profile (SD_{h:mm}) and high blood glucose index (HBGI). We did not observe statistically significant difference in low blood glucose index (LBGI). All results are presented in table.

Conclusions: Introducing the ‘Diabetics’ software in prandial insulin programming stabilized daily glucose profile particularly in terms of postprandial values. The bolus calculator ‘Diabetics’ software is a safety tool for self-managing children with diabetes.

Glucose variability parameters (Mean±SD)

	SD 2h after breakfast	SD 2h after lunch	SD 2h after dinner	Mean _T	SD _T	SD _W	SD _{h:mm}	HBGI	LBGI
GA	69,1±14,3	65,2±15,5	62,7±15,0	135,8±16,7	64,9±13,1	55,9±11,6	48,4±6,9	2,5±1,3	4,7±1,9
GB	80,0±18,3	81,3±20,5	82,5±23,9	153,7±25,5	79,4±18,4	68,7±15,8	54,7±10,5	4,4±2,5	4,7±1,9
p	0,03	0,00	0,00	0,01	0,00	0,00	0,02	0,00	0,72

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PS 086 Health care delivery 1

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Unnoticed shift in the normal range of glycated HbA_{1c} with TOSOH high performance liquid chromatographyC. Kloos¹, T. Heller¹, K. Böer², W. Hunger-Battefeld¹, G. Wolf¹, U.A. Müller¹;¹Internal Medicine, Klinikum für Innere Medizin III, Jena, Germany, ²IKCL, Germany.

Background and aims: Changes of the normal range of the HbA_{1c} makes long term follow up of studies dealing with the treatment quality of diabetes difficult. Additionally, patient care with the treatment target normoglycemia, e.g. pregnant patients with diabetes, uses as its standard the lower limit of the normal range of the local method. Since 2004 the HPLC method from TOSOH has been used and as of April 2010 the TOSOH G8 analyser UIN 20305. For the follow-up of a 20 year long-term study the normal range of the HbA_{1c} was checked every 5 years. In summer 2010, after retesting the normal range in 150 healthy non-diabetic volunteers, the normal range was found to have shifted.

Materials and methods: To specify the time of the shift, the HbA_{1c} values of 30568 patients with and without diabetes at a university out-patient department for endocrinology and metabolic diseases in the period from 2004 to 2010 were analysed. Women with gestational diabetes were excluded. The data was retrieved from the electronic patient data file EMIL[®].

Results: Compared to 2004 the mean normal HbA_{1c} tested in healthy volunteers increased from 5.24±0.33 (2004, n=107) to 5.64±0.25 (2009, n=150), (p<0.001). The mean HbA_{1c} values of non-diabetic patients from 2004 to 2010 also increased in absolute figures 0.4% (p<0.001): 2004: 5.4±0.41 (n=383), 2005: 5.36±0.39 (n=710), p(2004/5) n.s., 2006: 5.43±0.42 (n=960), p(2005/6)<0.001, 2007: 5.61±0.44 (n=1026) p(2006/7)<0.001, 2008: 5.52±0.40 (n=1054), p(2007/8)<0.001, 2009: 5.65±0.38 (n=1079), p(2008/9)<0.001, 2010: 5.67±0.37 (n=635), p(2009/10) n.s. In the period from 2006 to 2007 a shift in the normal range occurred. However, the mean HbA_{1c} values of diabetic patients did not show a consistent increase: 2004: 7.48±1.11 (n=2336), 2005: 7.65±1.24 (n=3740), p(2004/5)<0.001, 2006: 7.67±1.27 (n=3939), p(2005/6) n.s., 2007: 7.81±1.24 (n=4382), p(2007/8)<0.001, 2008: 7.55±1.13 (n=4110), p(2008/9)<0.001, 2009: 7.67±1.10 (n=3839), 2010: 7.72±1.05 (n=2330), p(2009/10)=0.05. In these patients, a shift cannot be found.

Conclusion: In spite of keeping the same HbA_{1c} analysing method and the same HbA_{1c} device of the same manufacturer a shift in the normal range occurred. This was detected due to an investigator/user driven check of the normal range in April 2010. This shift causes considerable changes in the normalisation results when the normal mean of the HbA_{1c} method is used for adjustment in long-term studies. Patient care is also a concern. For instance is one treatment target of diabetic woman during pregnancy to achieve a HbA_{1c} in the lower limit of the normal range. Using our normal range, this was supposed to be below 5.2% but found to be below 5.6%. On the other hand, the range for near normal glycemic control of patients using the DCCT as a model (equal or below 1.4 times of the normal mean of the local method) is either below 7.3% taking 5.2% as normal mean, or 7.8% using 5.6% instead. Until now no statement referring to the shift could be obtained by the manufacturer. To date, comparability of HbA_{1c} measurements during long term follow-up remains a problem.

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Development of new ways of measuring diabetes health in the Swedish National Diabetes Registry (NDR) taking a capability approachB. Palaszewski¹, F. Odegaard², S. Borg³, U. Lövgren¹, P. Roos⁴,S. Gudbjornsdottir¹;¹NDR, Gothenburg, Sweden, ²UWO, London, Canada, ³Swedish Institute for Health Econ (IHE), Lund, Sweden, ⁴RR Institute of Appl Econ, Lund, Sweden.

Background and aims: The aim of this project is to develop a method, as part of NDR, for measuring diabetes related health using biomedical and patient reported outcome measurements. In a health related capability approach (CA) the focus is on the individual well-being. We think of capability to perform functionings as a quality of life indicator, which can be improved from better understanding of how medical decisions affect living conditions.

Materials and methods: Using a newly developed questionnaire 4743 (801 type 1 and 3072 type 2) Swedish diabetes patients at 23 care centres were

asked to report their perceived diabetes related functioning for: health knowledge (self-care management, quality in communication with health care personnel); social life/working conditions (ability to overcome problems in daily life activities); confidence (anxiety for complications, access to health care). The response rate was 65%. The patients' clinical characteristics were representative for NDR and non responders did not differ in clinical characteristics from responders. All questions were measured on a 5 point Likert scale. Item Response Theory was used to translate ordinal data into scales. The resulting scales had good reliability, and scalability properties, as evaluated by Mokken Analysis were strong. Scores were calculated from the observed response pattern for each individual and each indicator. Scores were linked to indicators of the individuals' clinical characteristics. Indices reflecting various functioning a person potentially can achieve were obtained by using the 'ratio' between actual and potential functioning. In the construction of indices modern optimization methods, such as data envelopment analysis and Malmquist indices, were used. The constructed index was adjusted for differences in age, sex, onset age, and duration. An overall health index and two sub components (I Intermediate outcome of health services & II Health related daily life activities) were created.

Results: The results show variation among diabetes patients in all functioning indicators and the variation is much larger for sub index II compared to sub index I. Patients with Type 1 diabetes had more problems overcoming obstacles in social life and at work and more anxiety than those with type 2 diabetes (mean sub index II was 0.79 vs 0.84). Patients with type 2 diabetes on insulin had more problems in social life and at work and more anxiety compared to patients on diet or oral treatment (mean sub index II was 0.81 vs. 0.90). Results also show a non linear relationship between medical health variables and a person's potential functioning, i.e. non-proportional effects are observed for changes in medical variables. Furthermore, for some patients increased knowledge on how to live with diabetes has a greater positive impact on quality of life than improvements in some biomedical variables.

Conclusion: CA is very useful since it incorporates not only medical data on patients' health but also information on individuals functionings and capabilities. The method can also be used on an individual level, e.g. to evaluate the meeting between doctor/nurse and patient, as well as to identify patients with suboptimal health. Future work could result in an overall model for assessment of the provision of diabetes health care services, that can handle the complexity of provided services.

Supported by: BMS

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A systematic review of the impact of culturally-competent diabetes care interventions for improving diabetes-related outcomes in ethnic minority groups

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Background and aims: The provision of culturally-appropriate interventions to ethnic minority groups (EMGs) can confer important benefits, to the person with diabetes, their families and also bring about cost savings in every nation's healthcare system. However, recent systematic reviews on EMGs with diabetes have found limited evidence involving culturally-competent interventions. Because of this paucity and uncertainty regarding effective culturally-competent interventions, a systematic review with narrative analysis was undertaken, aimed to examine the effectiveness of culturally-competent interventions and innovations tailored to the needs of EMGs with diabetes globally.

Materials and methods: Four databases (Medline (NHS Evidence), CINAHL, Cochrane, DARE) and reference lists of retrieved papers were searched from inception to April 2010, including two NHS specialist libraries for diabetes, and ethnicity and health. In addition, we searched the Warwick Medical School Research Publications from 2006 to 2010. Paper selection and appraisal were conducted independently by two reviewers. The criteria for inclusion in the analysis were all effectiveness studies of any specified diabetes health-related intervention to any EMG within a majority population with diabetes. Data were collected on all reported outcome measures.

Results: Ten out of 271 studies were included. The heterogeneity of the studies required narrative analysis. Participants were recruited from 3 settings (primary care, hospital or community) and varied greatly such as South Asians, Russians, Hispanics, Turkish, etc. Study designs were varied and involved delivery by diverse range of health workers, e.g. certified diabetes edu-

cators, registered dieticians, podiatrist, multilingual link workers, bilingual health advocates, etc. Various outcomes were reported which included 7 self reported outcomes (e.g. satisfaction with diabetes education programmes), 9 assessed by staff (eye checks) and 4 objective validation (HbA1C). No study formally set out to systematically assess the cost effectiveness of their culturally-competent interventions or the quality of life. Although there were methodological limitations within the studies, findings suggest that interventions tailored to EMGs by integrating the element of culture into that intervention, (cultural and religious beliefs including linguistic and literacy skills), produced a positive effect. This was consistent in most of the ten studies.

Conclusion: We identified benefits in using culturally-competent interventions with EMGs with diabetes. Due to the mixed methodologies and outcome measures in the review, the data did not allow for convincing comparisons across countries, EMGs, or the type of interventions. However, further culturally-competent interventions are required and should include the cost-effectiveness evaluation which can easily influence diabetes service commissioners to decide on its implementation. Furthermore, there is need for culturally-competent structured education programmes which should include community leaders/companions of specific EMGs.

Supported by: West Midlands Strategy Health Authority, UK

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Age related quality of care; are younger, socially deprived patients disadvantaged?

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Background and aims: The 'epidemic' of diabetes is thought to be predominantly a problem of later life. Faced with the volume of demand services may struggle to deliver optimal care and there is the possibility that age related gaps in service quality may emerge. The English National Diabetes Audit (NDA) includes the majority of people with diagnosed diabetes. It assesses key annual processes of diabetes care, treatment target achievement rates and disease outcomes. Its comprehensiveness and scale enables general issues of deficient service delivery to be identified reliably. The 2009-10 data was examined for evidence of age related differences in care and/or outcomes.

Materials and methods: The NDA uses Primary Care, Specialist Care and Hospital Admission data collected electronically with an approved dataset. It is linked securely, confidentially and centrally in the NHS Information Centre. This annual national audit is now in its seventh year.

Results: In 2009-10 the English National Diabetes Audit included 1.9 million people, 83% of those with diagnosed diabetes in England. Between ages 10 years and 80 years Type 1 diabetes prevalence varies little (0.34-0.55 per cent). By contrast, Type 2 diabetes prevalence rises from 0.05% for ages 16-24 years and peaks at 14.44% for ages 70-84 years. In consequence those age <55yr are in the minority (24%). Also among those age <55yr Type2 diabetes is more than twice as common in the most deprived (3.0%) quintile of socioeconomic deprivation as in the least deprived quintile (1.3%); whereas above age 55yr there is no deprivation effect. Younger people less frequently received the nine core processes of care (all care processes were completed in only 20% age 16-24 years, 38% age 25-54 years but 55% age 55-84 years). Achieving the HbA1c ≤7.5% (58mmol/mol) treatment target was similarly less likely in younger people: thus for Type1 diabetes age <25yr it was <15% but in those age ≥55yr it was >30%; and for Type2 diabetes age <55yr it was <50% but in those age >70yr it was 70%. Appreciable numbers of complications start appearing in the 25-55 year age group after 10-20 years of diagnosed diabetes: 91% of DKA; 62% of End Stage Kidney Disease (ESKD) in Type1 and 15% of ESKD in Type2; 15% of Major amputations; and 12% of Myocardial Infarctions occur under age 55yr. All complications are 1.5-2.2 times more common in the more severe social deprivation quintiles.

Conclusion: The NDA confirms that the diabetes 'epidemic' is heavily weighted to older people. However, measures of care quality in younger people with both Type1 and Type2 diabetes are poorer than in older people. Furthermore social deprivation is associated with younger onset Type2 diabetes and complications risk. There should be a focus of effort on improving the effectiveness of services for younger people with diabetes who seem to be disadvantaged by comparison with their older counterparts.

Supported by: HQIP (Healthcare Quality Improvement Partnership)

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Quality of care from the perspective of patients with type 2 diabetes: a comparison between integrated and usual diabetes careG. Nijpels¹, A.A.W. van der Heijden¹, L.D. René¹, M.C. de Bruijne¹, C.A. Baan^{1,2}, S.D.M. Bot¹, T.L. Feenstra^{2,3}, G.A. Donker⁴, J.M. Dekker¹;¹The EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, ²National Institute for Public Health and the Environment (RIVM), Bilthoven, ³University Medical Center Groningen, ⁴Netherlands Institute for Health Services (NIVEL), Utrecht, Netherlands.

Background and aims: Integrated care using a patient-centred approach, can improve the interaction between the patient and health care professional, resulting in high-quality, satisfying consultations. We aimed to compare the perceived quality and continuity of diabetes care between patients receiving integrated diabetes care and usual care.

Materials and methods: 307 type 2 diabetes patients receiving integrated diabetes care by the Diabetes Care System and 463 type 2 diabetes patients receiving usual care participated in our study in the Netherlands. The Diabetes Care System coordinates the diabetes care between primary and secondary care. Patients have a central role in their care and are stimulated to make their own choices regarding treatment options and lifestyle behaviour. Self-management is stimulated and individual care plans are discussed with the patient. General practitioners receive feedback about their performance. Patients in the usual care group received care according to the current guidelines for type 2 diabetes by general practitioners only. A validated questionnaire, the QUality Of care Through the patients' Eyes questionnaire (QUOTE) for diabetes, was used to obtain information on patients' opinion about the quality and continuity of the diabetes care. Quality scores were calculated by combining the importance of aspects of care rated by the patient, with patients' experience with these aspects. We specified aspects of care that needed improvement and differentiated between patients' opinion on diabetes care by the general practitioner, the diabetes nurse and the dietician.

Results: In both groups, the discussion of aims and trajectory of treatment with the patient received good quality scores. With respect to the quality of care, most differences between the two diabetes care groups were seen in the care by the dietician, which was in favour of the Diabetes Care System. Regarding the continuity of care, many aspects were rated more favourable by patients treated by the Diabetes Care System compared to patients receiving usual diabetes care (Table).

Conclusion: Patients receiving integrated diabetes care experienced overall better quality of diabetes care and better continuity of care than patients receiving usual diabetes care.

Continuity of care scored by patients receiving integrated care and patients receiving usual care					
	Importance	Negative experience (%)		Quality score	
		integrated care	usual care	integrated care	usual care
It is important that... / It is my experience that...					
I received intensive support after diagnosis of diabetes	7.8 (2.1)	17.8	17.3	1.38	1.35
it is possible to consult a specialist in ophthalmology within 2 months	7.4 (2.2)	20.6	14.1*	1.58	1.01
I receive good education about self-control feet	7.2 (5.9)	5.9	20.5*	0.43	1.47
I receive a screening of the foot each year	7.4 (7.7)	2.3	19.7*	0.17	1.44
I receive a screening of the eyes each year	7.7 (1.9)	8.4	14.6*	0.65	1.12
I receive a screening of kidney function each year	7.6 (2.1)	31.4	41.5*	2.45	3.10
my blood pressure is assessed during each check-up	7.6 (1.9)	1.3	6.1*	0.10	0.46

Negative experience: proportion of patients that answered 'no' or 'not really' regarding the aspect of care.

* Indicates a significant difference ($p < 0.05$) in experience between the two groups.

A quality score between 0 and 1 indicates good quality. A quality score higher than 1 indicates low quality.

Clinical Trial Registration Number: ISRCTN66124817

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Glycaemic and risk factors control in type 1 diabetes: results from DIACAM 1 StudyJ. Sastre¹, P. Pines², J. Moreno³, B. Blanco⁴, S. Herranz⁵, R. Chamorro⁶, D. Calderon⁷, A. Marco¹, J. Lopez¹, DIACAM 1 study group;¹Endocrinology, Complejo Hospitalario de Toledo, ²Endocrinology, C H de Albacete, ³Endocrinology, CH Mancha Centro, Alcazar de San Juan, ⁴Endocrinology, H. Nra Señora del Prado, Talavera de la Reina, ⁵Endocrinology, H. U. Guadalajara ⁶Endocrinology, H. G. Ciudad Real, ⁷Endocrinology, H. Virgen de la Luz, Cuenca, Spain.

Background and aims: DIACAM 1 study was designed to investigate the clinical characteristics of a representative group of type 1 diabetic (T1D) population in Castilla La Mancha, a region in central Spain. The aim of this report is to evaluate metabolic control and to describe the prevalence of cardiovascular risk factors and the degree of control achieved.

Materials and Methods: This is an observational, cross-sectional, prospective and multicentre study of 1465 patients who received assistance in endocrinology clinics during 2010 as hospital outpatients. This cohort represents about 33% of the adult T1D patients in Castilla La Mancha. All reported patients were aged > 16 years at the time of the study and duration of diabetes was > 5 years. Diabetic patients underwent clinical and laboratory evaluation. Type of treatment was registered (insulin regimen, antihypertensive drugs and lipid lowering drugs). We consider intensive insulin regimen (IIR) when patients deliver insulin via multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII) with SMBG ≥ 3 day and self adjusting. Measurement of HbA1c was standardized to DCCT in all the laboratories. A multivariate logistic regression analysis was used to assess variables independently associated with good glycemic control ($\text{HbA1c} \leq 7\%$). P value less than 0,05 was considered statistically significant. Statistical analysis was done by SPSS 15.0

Results: Among 1465 patients, there were 48,5% women, mean age $39,4 \pm 13,5$ years and mean diabetes duration $19,4 \pm 10,6$ years. Mean HbA1c was: $7,8 \pm 1,2\%$. 26% of the patients reached $\text{HbA1c} \leq 7\%$, 62% had $\text{HbA1c} \leq 8\%$ and only 15% of the group had $\text{HbA1c} > 9\%$. 45% was treated with IIR (35% MDI and 9% CSII). In multivariate regression analysis: IIR (OR 2,56; 95% CI 1,97-3,34, $p < 0,01$), higher educational level (OR1,33; 95% CI 1,06-1,74 $p < 0,05$) and absence of smoking (OR 1,66 ; 95% CI 1,22-2,26 $p < 0,01$) were independently associated with $\text{HbA1c} \leq 7\%$. The prevalence of cardiovascular risk factors was (% CI95%): BMI > 30 15% (13,1-16,9), central obesity 26% (23,3-28,7), dyslipidemia 35%(32,5-37,5), hypertension 23% (20,8-25,2) and smoking 26% (23,7-28,3). 38% of the group was treated with lipid lowering drugs and 28% were receiving antihypertensive drugs. Lipids and blood pressure levels and degree of control are shown in table 1.

Conclusions: In this cohort of T1D patients glycemic control is still unsatisfactory, implementation of healthy attitudes (smoking cessation) and intensifying insulin treatment will improve glycemic control. Prevalence of cardiovascular risk factors is high in T1D although treatment goals are satisfactory.

Table 1	mean \pm sd	Targets (ADA 2010)	% (95%CI)
LDL-c (mg/dl)	102 ± 26	≤ 100	49 (46,3-51,7)
HDL-c (mg/dl)	58 ± 15	≥ 50	69 (66,5-71,5)
Triglycerides (mg/dl)	89 ± 106	≤ 150	91 (89,5-92,5)
Systolic BP (mmHg)	126 ± 16	≤ 130	71 (68,6-73,4)
Diastolic BP (mmHg)	73 ± 10	≤ 80	81 (78,9-83,1)

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Evaluation of the benefits of a regional pre-pregnancy care program in women with type 1 and type 2 diabetes: a prospective study

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Background and aims: Diabetes is the commonest medical problem in pregnancy with increased risks for both mother and infant. To decrease perinatal morbidity and mortality to that of the background population and improve maternal morbidity women need to attend pre pregnancy care (PPC) to optimize their glycaemic control, use folic acid, review medications and screen

for diabetic complications. As part of our ATLANTIC DIP program we are undertaking a single arm prospective study to compare pregnancy outcomes in women who attend (attendees) PPC to those who decline an invitation to attend (Non-attendees).

Materials and methods: Through ATLANTIC DIP and DIAMOND databases, hospital and primary care registers, 541 women of child bearing age with Type 1, Type 2 Diabetes or IFG/IGT following GDM were identified. Patients were contacted by invitation letter and followed by a phone call to confirm their interest in attending one of 4 PPC clinics established in the region. Consent was obtained at their first visit. The PPC intervention centers around pregnancy specific education, dietary advice, glycaemic targets, review of medications, review of complications, rubella status and commencement of folic acid. Data on maternal and infant outcomes of pregnancy and glycaemic control are collected real time on DIAMOND.

Results: To date 131 women (24.2%) have consented and attended for PPC 71 Type 1 and 60 Type 2+IGT/IFG). Of these 131 women there are 67 confirmed pregnancies. All received folic acid (100%) and mean HbA1c was 6.6% (5.2–8.5) prior to conception with >80% attendees with HbA1c 7%) and uptake of folic acid in only 43% (1). 15 invited but declined PPC patients had 15 confirmed pregnancies with a mean pre pregnancy HbA1c of 8.1% (5.2–11.7) and folic acid uptake of 27%. 52 pregnancies have now delivered. The miscarriage rate was 7.0% and admission to neonatal unit (NNU) 19% in attendees compared to 13% and 40% respectively in non-attendees. The background population rates are 15% and 11% respectively. The figures for miscarriage and NNU admission in women receiving PPC is significantly better than our previous reported rates of 22% and 48% respectively (1). The caesarean section rate was lower in attendees (48%) compared to non-attendees (60%). There were no differences in stillbirth rates between groups. No congenital anomalies reported.

Conclusion: The increased uptake of folic acid and improvement in glycated haemoglobin levels are encouraging. This is translated into a decrease in miscarriage and an increase in take home baby rates. In addition caesarean sections and admissions to NNU care are less in attendees resulting in financial savings. The low rate of uptake is of concern and requires further investigation through focus groups.

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The impact of the UKPDS Risk Engine on cardiovascular risk management in patients with type 2 diabetes mellitus in general practice

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Background and aims: It is not clear whether prediction rules, like the UKPDS risk engine, have added value to cardiovascular risk management of type 2 diabetes mellitus (T2DM) patients in general practice. In this study, we want to investigate this by answering the following research questions: Which patient characteristics are associated with under- or overestimation by the general practitioner (GP) of the cardiovascular risk of T2DM patients? What is the impact of the UKPDS risk engine on prescription of medication by the GP and on cardiovascular risk factors?

Materials and methods: A 12-months observational study in 993 patients on the list of 117 GPs. Risk factors for cardiovascular disease were collected at baseline and after 12 months. GPs had to estimate the cardiovascular disease risk themselves and by using the UKPDS risk engine, and subsequently had to state if they had adjusted the patient's medication. Generalised Estimating Equation (GEE) modelling was used to identify independent predictors of under- and overestimating patient's cardiovascular risk, taking into account clustering of patients within GPs. The impact of the UKPDS risk engine was assessed by measuring the differences in medication adjustments and changes in metabolic variables between the over- and underestimated group during 12 months.

Results: The mean difference between the cardiovascular disease risk estimated by the GP and calculated with the UKPDS engine was -0.36 (95% CI -1.24;0.52), ranging from 52% underestimation to 72% overestimation. Age (year), male gender, HbA1c (%), total cholesterol (mmol/l), HDL-cholesterol (mmol/l) and current smoking were associated with underestimation of cardiovascular risk by the GP (respective ORs (95% CI): 1.14 (1.11;1.18), 7.45 (4.61;12.04), 1.54 (1.18;2.00), 2.16 (1.70;2.74), 0.05 (0.02;0.11), 2.19 (1.28;3.75)). HbA1c was associated with overestimation of cardiovascular risk by the GP (OR 1.33, 95% CI 1.07;1.65). Patients with an underestimated cardiovascular risk received significantly more medication adjustments com-

pared with overestimated and accurately estimated patients. More improvements in cardiovascular risk factors after 12 months were seen in the underestimated group.

Conclusion: Age, gender, HbA1c, total cholesterol, HDL-cholesterol and smoking were associated with underestimation, and HbA1c was associated with overestimation of cardiovascular risk by the GP. The UKPDS risk engine is of added value for cardiovascular risk management in T2DM patients in general practice and can improve medication prescription and cardiovascular risk factors in this high-risk target group.

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Pursuing perfection in the diabetes review clinic

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Background and aims: Timely, thorough and holistic evaluation of the patient with diabetes, screening for complications and strategies to prevent future problems is the mainstays of the diabetes review clinic. However, these types of clinics have varying formats and content depending on their organisation, efficiency and locale. We wished to prospectively assess how successful we were being at asking about and accurately recording data relating to two important aspects of ongoing diabetes; neuropathy symptoms such as erectile dysfunction (in men) and pregnancy plans (in women of child bearing age). Both are stressed as significant in the latest NICE guidelines for diabetes care.

Materials and methods: We collected anonymised information about the content of the diabetes review consultation through two main processes. Firstly, a post-consultation neuropathy screening questionnaire which was given to patients in the manner of an exit poll to collect information about whether the patient has been asked about various neuropathy symptoms including erectile dysfunction (the clinician being blinded to this occurrence). Secondly, data from the diabetes database DIABETA 3 was mined to reveal whether young females had their pregnancy intentions discussed (there being a forced choice checkbox within the computer programme) and subsequent pre-conception counselling arranged for those who were actively planning to become pregnant.

Results: With regards to erectile dysfunction we discovered that only 21% of men with diabetes attending the annual review clinic were asked about this particular symptom and overall that diabetologists fared poorly in enquiring about all the different potential signs and symptoms of neuropathy. With regards to pregnancy intentions and arranging follow up pre-conception planning, only 12% of female patients (of child bearing age, 16–45 yrs) were asked about this.

Conclusion: It would appear as though the diabetes review clinic is sub-optimal in its ability to explore and screen for signs and symptoms of neuropathy, such as ED in men, as well as crucially finding out about pregnancy plans in young females. The former is significant because of its impact on quality of life and the second is potentially devastating because of the consequences of poorly controlled diabetes in the ante-natal period. Given that 'prevention is better than cure' it would seem germane to look at better ways of information gathering in the context of the diabetes review clinic. To this end we have designed a pre-consultation neuropathy screening tool which is in the process of being validated and a more rigorous approach to enquiring about pregnancy plans in women of child bearing age with diabetes. New modalities such as 'Care Planning' may well be the way forward.

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Hypoglycaemia in a hospital setting: a survey on nursing practices from a tertiary care hospital in India with a case for the role of diabetes nurse educator

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Background and aims: Hypoglycemia is a known complication of insulin therapy. Patient related factors as causes of hypoglycemia have been widely studied with relative paucity of studies on nursing practices contributing to hypoglycemia and its management. We conducted a survey at a single tertiary care hospital on the ongoing practices for hypoglycemia management our corrective measures based on the observations and a repeat survey.

Materials and methods: A survey was done on hospital records from Dec 2008 to March 2009 for cases of hypoglycemia (BG < 70 mg/dL) on insulin

therapy. Reaction time was noted, defined as time between the first documentation of hypoglycemia and any oral or parenteral intervention. A questionnaire survey was done on nursing staff to check on understanding of hypoglycemia management. Based on initial assessment, corrective intervention was done. A repeat audit was done in the year 2010 on case records over four months duration for a pilot comparison.

Results: There was lack of uniformity of hypoglycemia management across wards with long reaction time (6–12 minutes) for managing 41 instances of hypoglycemia. Possible sources of overdosing included misinterpretation of prescription notes, and surprisingly a lack of appropriate understanding of U40 and U100 insulin's specific syringes. Corrective measures were put in place by introducing hypoglycemia protocol, glucose insulin chart, hypoglycemia tool kit for all wards, availability of single strength insulin (U100) and regular education to nursing staff on hypoglycemia management. In the second audit, there were 25 episodes of hypoglycemia with reaction time of 2–4 minutes with no discrepancy between physicians' prescription and nursing administrations.

Conclusion: Although not a controlled study to assess outcome measures of intervention, the pilot study revealed key observations of lack of uniform practices of hypoglycemia management, prolonged reaction time, and some of the old errors of insulin IU and syringe mismatch. All these factors are readily avoidable by continuous education to nursing staff providing a case for the role of diabetes educator nurse at all hospitals in India and abroad.

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Impact of medication treatment on mortality and readmission in patients with hyperglycaemia prior to and during hospitalisation

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Background and aims: Little is known about the impact of medical management of diabetes before hospitalization on in-hospital mortality and readmissions.

Materials and methods: Using a hospital database linked to outpatient data with 73,977 admissions between July 1, 2003 and December 31, 2009, we identified 18,078 patients with a clinical encounter 12 months before and after an "index" admission (IA). To gain understanding of impact of pre-admission medical treatment on hospitalization outcomes, we evaluated diabetes (DM) status and use of antidiabetic medication pre-admission (PA) and during IA and further examined the association of mortality with anti-diabetes, antihypertensive and antilipidemia medication treatment.

Results: At IA mean age was 50 ± 16 years and 52% were female. At IA 25% had pre-existing DM (Group A), 7% were newly diagnosed (Group B), 27% were not DM but with hyperglycemia (any BG > 140 mg/dl) (Group C) and 41% were not DM with no hyperglycemia (Group D). During IA, nearly 60% had hyperglycemia or DM diagnosis and 349/18,078 (1.9%) died. Group C had the highest mortality during IA, 216/349 (62%), a 2.8 fold increase compared to Group A and B DM patients ($p < .0001$). Patients receiving insulin or oral hyperglycemic medication (OHM) either PA or during IA experienced lower mortality than those not receiving insulin or OHM, ($p < .0001$, $p < .007$). Less than half of Group A patients were receiving antihypertensive and antilipidemia medication treatments PA. In Group A and B patients, patients who were not taking these therapies PA had a 2- and 3-fold higher mortality risk than those patients who were. Mortality in patients in Group C and D was not affected by these medications.

Conclusion: This study showed that mortality risk was lower in patients receiving Insulin and or OHM either PA or during IA across all groups, suggesting that hyperglycemia treatment offers a mortality benefit. The presence of drugs commonly associated with CV protection in the outpatient setting might also offer some protection for hospitalized patients. Further analysis of mortality risk adjusting for comorbidity at IA will be required to confirm this relationship.

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Impact of providing people with type 2 diabetes with their actual risk for five diabetes complications: a pilot study

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Background and aims: Many individuals feel hopeless and helpless to manage their diabetes. These individuals vastly overestimate their risk of developing complications, and underestimate their ability to reduce their risks. Previous research suggests that providing people with their actual risk of complications, reduced their distress and increased their engagement in risk reduction information. Therefore, this feasibility study sought to determine whether providing people with poorly controlled type 2 diabetes with their actual risk for the complications of diabetes (heart attack, stroke, blindness, amputation, kidney disease), would lead to improved control and reduced psychological distress.

Materials and methods: Individuals with poor control of their diabetes (HbA1c > 8.0; plus one of BP > 140/80, smoker, Total Cholesterol > 4.0) were recruited from GP practices in three rural communities in Western Australia. Baseline assessment of lipids, HbA1c, BP, depression (CESD) and diabetes distress (PAID) were completed, and individuals were randomized to receive personalized complication specific risk information alone, provided by the Mellibase risk engine, or risk information with a 3 monthly structure goal setting and telephone support program. At 9 months after baseline assessment, individuals completed a second assessment of all outcome measures.

Results: 54 individuals were randomized across the two groups. There were no significant differences between control and intervention groups at baseline or completion of the study, for HbA1c, lipids, BP, depression or diabetes distress. However, both groups evidenced significant reductions in HbA1c (Baseline HbA1c= 8.8; SD=0.9; Post HbA1c=8.2; Sd =1.4; $p<.001$), and for those who had cholesterol above target at baseline (Baseline Total Chol=5.3 SD=1.0; Post = 4.5; SD=1.2; $p<.01$). Participants also reported less distress (Baseline PAID= 16.6 SD=14.4; Post PAID=13.8;SD 11.8; $p<.05$), but there was no effect on depression.

Conclusion: Providing people diabetes with personalised complication specific information about their risk of microvascular and macrovascular complication may facilitate more pro-active management of diabetes and reduced diabetes related emotional distress.

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Does a PDA improve knowledge about treatment choices for patients with type 2 diabetes mellitus when making decisions about starting insulin? Quantitative analysis from the PANDAs study

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Background and aims: Type 2 diabetes (T2DM) is a common illness, affecting about 2.8 million people in England in 2010. Despite lifestyle changes and glucose lowering drugs, a significant number of these people continue to have poorly controlled blood sugar due to disease progression. As poor glucose control leads to an increased risk of developing complications (eg stroke, coronary heart disease, renal problems), doctors will eventually recommend insulin therapy. However, uncertainty about the treatment choices may lead to patients delaying their decision about starting insulin. Using quantitative data from the PANDAs study we will explore how knowledgeable patients are about their treatment choices.

Materials and methods: PANDAs (Patients ANd Decision Aids) is a cluster randomised controlled trial (RCT) that was conducted across South Yorkshire. Its aim was to establish whether the use of a patient decision aid improves the decision quality and health outcomes of patients with T2DM who are considering insulin therapy. The decision aid provides the patients with key facts about their T2DM as well as clarifying their own values on management of their diabetes, in order to help them make a decision about their treatment. Patients recruited into the both arms of the study were asked to complete a questionnaire before and after their consultation with their usual Health Care Professional, part of which explored their knowledge about treatment choices whilst patients in the intervention arm also completed a decision aid prior to their consultation.

Results: A total of 175 patients were recruited into the study; 95 intervention and 80 control. The mean age was 65 years (\pm 10 SD). 96 participants were male, and the mean duration since diagnosis of diabetes was 8 years (\pm 4 SD). When asked if they knew which treatment choice has the greatest chance of lowering their blood sugar, 23/80 control and 49/95 intervention chose the correct answer ($p=.002$ chi square). When asked about number of 'hypo's they might experience in a year, if taking insulin, 4/76 control and 77/95 intervention chose the correct answer, ($p=.000$ chi square), and when asked how much weight they might gain in a year, if taking insulin, 4/75 control and 67/95 intervention chose the correct answer ($p=.000$ chi square). When asked about their risk of getting complications in 5 years if they were to go onto insulin, 4/80 control and 25/95 intervention chose the correct answer, ($p=.000$ chi square). There was no significant difference between the two groups when asked about which treatment would lower their complications.

Conclusion: Patients in the intervention group are more knowledgeable about their treatment choices after using the decision aid compared to the patients in the control arm. Decision Aids, such as the PANDAs decision aid can help to improve the knowledge of patients about their illness and treatment options which can help them make an informed decision about their future treatment.

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DiabetesE: an innovative and effective alternative to peer review for assessing and benchmarking the quality of diabetes service provision

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Background and aims: DiabetesE is an online self assessment tool which measures and benchmarks the quality of diabetes service provision and drives continuous quality improvement. Users can update their answers whenever they wish and data is analysed instantly. DiabetesE was launched in 2003, in England, to support the implementation of the Diabetes National Service Framework and is one of a suite of complementary tools which operate under the auspices of the National Diabetes Information Service. The aim of DiabetesE is to provide a methodology for undertaking a comprehensive assessment of the quality of diabetes services in England to inform local commissioning and national priority setting and to encourage service improvement.

Materials and methods: Two new questionnaires were developed, one focusing on diabetes services commissioning and another on specialist diabetes service provision. A review of national diabetes related policy and clinical literature was undertaken and an expert reference group of service users, clinicians and managers advised on the draft questions. These were grouped into 3 commissioning and 12 specialist diabetes provider modules. An interactive process was used to refine and agree the final set of questions and for each question a supporting rationale, based on current evidence, was built into DiabetesE via electronic links. The expert reference group ranked the modules and questions so that a scoring and weighting system could be devised and applied, enabling the automatic generation of scores and of prioritised recommendations for encouraging service improvement. Users can compare their results locally, regionally and nationally and view National Diabetes Audit outcome data. The questionnaires were piloted in summer 2010 and launched in November 2011.

Results: By 31st January 2011, 107 commissioning organisations and 124 specialist diabetes providers had assessed their services. Modules are scored as a percentage (100% = best possible score). The Diabetes in Pregnancy module had the highest mean score (82%). Although the Children and Young People with Diabetes module was the next highest scoring (72%), 4% of services scored under 25%. The majority of services scored over 50% in all the modules, with the exception of Inpatient Management of Active Diabetic Foot Disease, which had the lowest mean score (45%) and for which only 37% of specialist diabetes providers scored over 50%, with 17% scoring under 25%. Kidney Screening and Management was the second lowest scoring module (59%), with 34% of providers scoring under 50% and 5% scoring below 25%. In one of the commissioning and four of the specialist diabetes provider modules, a few services scored 100%, notably for Staff Development, where 8% of specialist diabetes providers achieved this score.

Conclusion: DiabetesE data captured at the end of January 2011 provides a detailed snapshot of the quality of diabetes care in England. The variation between module scores indicates that some aspects of diabetes service provision are better than others, but significant work remains to bring all services up to national standards. Whereas peer reviews capture information about service provision at a specific time, DiabetesE data is updated instantly and on an ongoing basis. Progress in improving all aspects of diabetes care can therefore be quickly and easily monitored through implementation of the DiabetesE recommendations and use of the integral benchmarking functionality, making DiabetesE an effective alternative to peer review.

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Optimal type 2 diabetes management including benchmarking and standard treatment: Portuguese data for the OPTIMISE study

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Background and aims: Current medical practice is not always successful in achieving therapeutic goals for glycemia, blood pressure and cholesterol. The benchmarking technique can improve diabetes treatment by giving feedback to physicians on the achievement of pre-set targets according to current guidelines. The OPTIMISE study, carried out in 6 European countries, aims at demonstrating that the use of benchmarking improves quality of care for patients with type 2 diabetes. This abstract describes Portuguese results.

Materials and methods: Participating investigators were randomly assigned to a benchmarking or to a control group in a ratio of 3:1. All investigators received feedback on each patient's risk factors. The benchmark group also received an anonymous comparison with their colleagues. The primary end-point was the percentage of patients achieving pre-set targets according to European guidelines (2007) for HbA_{1c} (7%), low-density lipoprotein cholesterol (LDL-C (<100 mg/dl) and Systolic Blood Pressure (SBP <130 mmHg), after 12 months.

Results: A total of 15 investigators recruited 185 patients, 134 for the benchmark group and 51 for the control group. Patients' baseline characteristics were not significantly different. Patients had a median age of 61.4 years (minimum 37.1 and maximum 92.3) and 50.8% were women. At the time of study inclusion, a mean 11.7 years had elapsed since diagnosed with diabetes. The most commonly used diabetes medication was biguanides (78.4%), with biguanide-sulfonamide being the most frequent combination (22.8%). Statins were the most commonly used class of lipid lowering medication (89.9%) and sartans (61.6%) and diuretics (57.2%) were the most frequent hypertensive therapies. In general, the number of patients who reached each clinical target increased between baseline and 12 months. The target for SBP was achieved by 23.2% (benchmarking 23.2%; control 23.3%) of patients at baseline and by 25.9% (benchmarking 22.1%; control 33.3%) after 12 months. At baseline 27.7% of patients were on target for HbA_{1c} (benchmarking 28.1%; control 26.9%) and after 12 months this increased to 32.2% (benchmarking 30.9%; control 34.8%). The number of patients reaching LDL-C target levels increased from 41.6% (benchmarking 40.3%; control 45.5%) at baseline to 45.8% (benchmarking 46.7%; control 43.6%) after 12 months. All three targets were achieved by 3.4% of patients (benchmarking 3.79%; control 2.38%) after 4 months and by 3.6% after 8 months (benchmarking 3.91%; control 2.44%). After 4 and 8 months the amount of patients achieving all three targets was higher for the benchmarking group. At the 12 month point this decreased to 1.5% (benchmarking 1.04%; control 2.56%).

Conclusion: Despite the small differences between groups, the amount of patients achieving each of the evaluated targets increased between baseline and 12 months. Between the benchmarking and the control groups, more patients in the benchmarking group achieved the three targets after 4 and 8 months of follow-up. However the opposite was shown after the 12 month period. The achievement of therapeutic goals for type 2 diabetes in Portugal is still suboptimal and may be influenced by numerous factors. In order to improve quality of care for Portuguese diabetes patients, the implementation of interventions such as benchmarking is still necessary.

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Perioperative diabetes care: gap between recommended care and current practice

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Background and aims: Optimal perioperative diabetes care reduces mortality, infection rates and length of hospital stay. Daily practice however, shows that optimal perioperative diabetes care is very often not achieved. To identify key problems in the delivery of optimal perioperative diabetes care, we first defined optimal perioperative diabetes care by developing quality indicators, and subsequently measured current quality of care.

Materials and methods: To define optimal perioperative diabetes care, a systematic RAND-modified Delphi method was used to derive a set of key recommendations from international literature and guidelines on perioperative diabetes care. The resulting set of key recommendations was systematically transformed into 17 indicators on professional performance, 9 indicators on organizational structure, and 4 indicators on patient outcome. A focus group interview in diabetic patients on their experiences with previously received perioperative diabetes care, yielded 12 complementary indicators on patient-oriented quality. To measure current quality of care, indicator performance was assessed in 400 diabetic surgical patients from six Dutch hospitals. Diabetic patients were included if they had abdominal surgery during general anesthesia, heart surgery or large joint orthopedic surgery with a minimum operative time of one hour in the period march 2009-march 2010. Professional performance was assessed by medical record search. Organizational structure was assessed by questionnaire. Selected patients received a questionnaire on patient-oriented quality.

Results: Regarding professional performance, medical records revealed information on preoperative glycaemic control in only 55% (24-87%) of patients. Preoperative information on macrovascular complications was retrieved in 92% (82-96%) of patients, whereas preoperative information on microvascular complications was recorded in only 28% (6-78%) of patients. Recordings of intraoperative blood glucose values could be retrieved in merely 27% (2-54%) of patients. Regarding organizational quality, protocols concerning perioperative diabetes care were present in all six hospitals. A multidisciplinary team in which all professionals involved in perioperative diabetes care were represented was absent in all six hospitals. Regarding patient outcome, 44% (41-52%) of postoperatively measured blood glucose values on the surgery ward were between 6-10 mmol/l. Regarding patient-oriented quality, only 41% (35-47%) of patients received information on diabetes medication regime in the perioperative period.

Conclusion: This study identified multiple opportunities for improvement of perioperative diabetes care. To improve perioperative diabetes care, we performed in-depth interviews with professionals involved in the perioperative diabetes care process. These revealed a lack of knowledge, lack of agreement on responsibilities of the various involved care takers, lack of insight into own performance and the perception that perioperative diabetes care has low priority. To improve perioperative diabetes care, a multifaceted strategy to address these key problems including education, multiprofessional establishment of a perioperative diabetes care protocol, feedback on performance, and patient oriented interventions is needed.

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Approaches to insulin intensification in the A_{1c}chieve observational study

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Background and aims: Current evidence substantiates various strategies for intensifying an insulin regimen as type 2 diabetes progresses. Patient characteristics, HbA_{1c}, previous therapy and regional differences may strongly influence physicians when intensifying an insulin regimen.

Materials and methods: Data from A_{1c}chieve - a large, global, observational study conducted across seven regions (China; South Asia; East Asia; North Africa; Middle East/Gulf; Latin American and Russia) - were used to explore the relationship between therapy before the study start and choice of subsequent insulin regimen intensification under routine clinical conditions throughout.

Results: Three main groups (total n=4237) were identified: basal±oral glucose-lowering drugs (OGLDs) switched to mealtime+basal insulin (MB) [n=470]; premix±OGLDs switched to MB [n=979]; and basal±OGLDs switched to premix±OGLDs [n=2788]. Demographic characteristics were similar between the basal-to-MB, premix-to-MB and basal-to-premix groups: mean age 54.2 (SD 11.9), 52.7 (14.0) and 57.4 (11.2) yr; duration of diabetes was 12.4 (7.4), 12.8 (7.9) and 12.6 (8.0) yr; and HbA_{1c} 9.7 (1.8), 9.5 (1.7) and 9.6 (1.8)%, none of which predicted regimen choice. Likewise, morning fasting plasma glucose (FPG) was similar before intensification across all groups (range 10.3-10.8 mmol/l), as was postprandial glucose (PPG) (post-breakfast) 14.3-15.1 mmol/l. Overall hypoglycaemia was most frequent in the premix-to-MB group compared with the basal-to-MB and basal-to-premix groups (28.4, 24.5 and 14.1%), as was major hypoglycaemia (7.4, 4.8 and 2.1%). Insulin dose increment was highest in the basal-to-MB group compared with the basal-to-premix and premix-to-MB groups (31.6 to 51.4 vs 29.9 to 36.9 and 53.6 to 57.8 U/day). There were strong regional differences in choice of insulin regimen, with difference in choice of starting regimen affecting subsequent approaches to intensification (Table). Generally, people treated with basal insulin were more likely to be intensified to a premix insulin regimen than to a mealtime+basal regimen. In China, premixed insulin is more commonly used than basal or prandial, the reverse being true in Russia and Latin America.

Conclusion: Mean HbA_{1c} at baseline, irrespective of insulin regimen choice, predicted that treatment intensification was overdue. Regional dif-

ferences rather than patient characteristics or disease duration are predictors both of regimen when beginning insulin and subsequent pathway of intensification.

Regional distribution of people starting insulin and those intensifying within 6 months				
Region	All patients	From basal insulin		From premix
	Insulin starters (n)	To meal+basal insulin (n [%])	To premix insulin (n [%])	To meal+basal insulin (n [%])
China	10929	30 (0.3%)	235 (2.2%)	152 (1.4%)
South Asia	22373	69 (0.3%)	516 (2.3%)	105 (0.5%)
East Asia	9962	88 (0.9%)	781 (7.8%)	36 (0.4%)
North Africa	3980	77 (1.9%)	285 (7.2%)	118 (3.0%)
Middle East/Gulf	14216	159 (1.1%)	601 (4.2%)	537 (3.8%)
Latin America	1096	26 (2.3%)	109 (9.9%)	5 (0.5%)
Russia	3042	70 (2.3%)	261 (8.6%)	26 (0.9%)
Total	65685	519 (0.8%)	2788 (4.2%)	979 (1.5%)

Clinical Trial Registration Number: NCT00869908

Supported by: Novo Nordisk

1002

Baseline characteristics of the First Basal Insulin Evaluation in Asia (FINE Asia) study population

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Background and aims: First Basal Insulin Evaluation in Asia (FINE Asia), a multinational, 6-month, prospective observational study, assessed the use of basal insulin in insulin-naïve Asian patients (aged ≥ 20 years) with type 2 diabetes mellitus, uncontrolled ($A1C \geq 8\%$) on oral antihyperglycemic drugs (OADs). Here we compare baseline characteristics across 11 Asian countries.

Materials and methods: Basal insulin was initiated with or without concomitant OADs; all treatment choices were at the physician's discretion. A total of 2921 patients were enrolled at baseline; 2679 patients with both baseline and 6-month HbA_{1c} values were included in the analysis.

Results: Demographic characteristics and insulin regimen at baseline are shown in the table. Most patients initiated insulin therapy with insulin glargine. Across the countries/region, mean duration of diabetes ranged from 6.3 to 11.5 years and mean baseline HbA_{1c} ranged from 9.4% to 10.5%. The prevalence of microvascular disease ranged from 14% to 39%, coronary artery disease from 9% to 21%, and dyslipidemia from 49% to 84%.

Conclusion: The results indicate that treatment intensification and the initiation of insulin are delayed in patients in these countries, irrespective of international, regional, and local treatment guidelines, and that management of other metabolic risk factors is delayed as well.

	China n = 491	India n = 681	Korea n = 291	Pakistan n = 139	SE Asia ^a n = 212	Taiwan n = 417	Thailand n = 448
Age, y	56.0	54.9	57.6	50.8	56.6	60.1	56.3
Weight, kg	69.7	71.9	65.0	76.6	61.3	65.5	65.6
BMI, kg/m ²	25.5	27.6	25.0	27.9	24.2	25.4	26.4
Duration of diabetes, y	6.3	9.8	10.7	8.6	9.5	11.5	9.1
Duration of OAD use, y	5.8	9.2	9.2	7.8	8.4	11.5	9.0
HbA_{1c} , %	9.4	9.4	9.7	10.1	10.5	10.2	10.2
FPG, mg/dl	185	211	205	205	230	226	216
Insulin regimen, %							
Glargine	61.9	94.3	90.7	100	53.3	73.6	55.1
NPH	37.5	5.0	9.3	-	42.9	12.5	44.9
Detemir	-	0.7	-	-	-	13.4	-
Other	0.6	-	-	-	3.8	0.5	-

^aBangladesh, Hong Kong, Indonesia, Singapore, Vietnam.

FPG, fasting plasma glucose

Values are mean or %

Supported by: sanofi-aventis

1003

Initiation of basal insulin in patients with type 2 diabetes: national differences in glycaemic control after 6 months in a subanalysis of the FINE Asia database

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Background and aims: FINE Asia is a prospective, observational registry undertaken to evaluate basal insulin initiation in patients in Asia with type 2 diabetes mellitus who were inadequately controlled by oral antihyperglycemic agents. This subanalysis compared findings by individual participating countries.

Materials and methods: The primary efficacy end point was change in HbA_{1c} from baseline to Month 6 after basal insulin initiation. Secondary end points included change in fasting blood glucose (FBG) from baseline to Month 6, HbA_{1c} and FBG responses rates, change in insulin dose, and hypoglycemic events.

Results: The study enrolled 2921 patients from 11 Asian countries (countries with small patient numbers are pooled as Southeast Asia); 2679 patients with both baseline and 6-month HbA_{1c} values were included in the analysis. Overall, HbA_{1c} decreased from $9.8 \pm 1.6\%$ to $7.7 \pm 1.4\%$ at Month 6 after starting basal insulin (NPH insulin, insulin glargine, or insulin detemir); 33.7% of patients reached $HbA_{1c} < 7\%$. Glycemic control at Month 6 varied greatly by country (Table), with a low of 10.6% and a high of 75.4% of patients in Taiwan and China, respectively, reaching the HbA_{1c} target. The increase in insulin dose ranged from 0.5 U in Pakistan to 6.0 U in Thailand. Rates of hypoglycemia also varied from 2.2% of patients in India to 15.9% of patients in China experiencing at least 1 event.

Conclusion: National data from the FINE Asia study show widely varying degrees of glycemic control in patients depending on their country, with China having the highest percentage of patients achieving $HbA_{1c} < 7\%$ but also having the highest rate of hypoglycemia.

Country	n	Baseline HbA _{1c} % (SD)	Δ HbA _{1c} % (SD)	Patients With HbA _{1c} < 7, %	Patients Reaching FBG < 110 mg/dl, % (SD)	Δ Total Daily Insulin Dose, U (SD)	Patients With Hypoglycemia, %
China	491	9.4 (1.6)	-2.6 (1.5)	75.4	54.8	1.2 (4.6)	15.9
India	681	9.4 (1.2)	-2.3 (1.2)	59.8	46.1	1.7 (5.1)	2.2
Korea	291	9.7 (1.6)	-1.6 (1.8)	18.2	20.6	0.8 (4.8)	7.9
Pakistan	139	10.1 (1.3)	-2.6 (1.6)	59.0	74.1	0.5 (3.1)	10.8
Taiwan	417	10.2 (1.7)	-1.3 (2.0)	10.6	26.6	2.4 (9.0)	5.8
Thailand	448	10.2 (1.9)	-2.1 (2.0)	24.8	27.9	6.0 (8.6)	8.7
SE Asia*	212	10.5 (1.9)	-2.5 (2.2)	34.4	25.0	2.8 (7.0)	8.5

*Bangladesh, Hong Kong, Indonesia, Singapore, and Vietnam

Supported by: sanofi-aventis

1004

Self-rated health predicts overall mortality in patients with type 2 diabetes

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Background and aims: Self-rated health scores have shown to be predictors of vascular events and major complications in diabetes patients. We aimed to investigate whether low self-rated health is associated with increased mortality in patients with type 2 diabetes (T2D).

Materials and methods: Self-rated health was assessed with four answering alternatives in self-administered questionnaires in 3257 patients (mean ± SD age was 55.8 ± 7.6 years and 41.6% women) with confirmed diagnosis of T2D. Enrolment took place between 1992 and 2000 in four centers (Bilthoven, Heidelberg, Potsdam, Umeå) in a sub-cohort nested in the European Prospective Investigation into Cancer and Nutrition. Causes and dates of deaths were ascertained using record linkages with boards of health, death indexes and cancer registries or by follow-up mailings and subsequent inquiries to municipal registries, regional health departments, physicians, or hospitals. We used Cox proportional hazards modeling to estimate hazard ratios (HRs) with 95% confidence intervals (95% CIs) associated with self-rated health controlling for age, sex, BMI, physical inactivity, hypertension, hyperlipidemia treatment, smoking, previous myocardial infarction and cancer diagnosis.

Results: During follow-up (mean follow-up ± SD was 8.6 ± 2.3 years), 344 deaths (241 men/103 women) occurred including 40.4% cardiovascular deaths. In a multivariate model, patients rating their health as “moderate” or “poor” were associated with increased mortality (HR 1.45, 95% CI 1.16–1.81, Figure 1) compared to patients rating their health as “excellent” or “good”. As a comparison, the HR for smoking was 1.58 (95% CI 1.23–2.03) in the same model. We found no indication of heterogeneity between centers (see Figure 1).

Conclusion: Low self-rated health is associated with increased mortality in patients with T2D after controlling for established risk factors. Whether this association can be extended to a more specified mortality outcome such as cardiovascular deaths, needs to be investigated in larger studies.

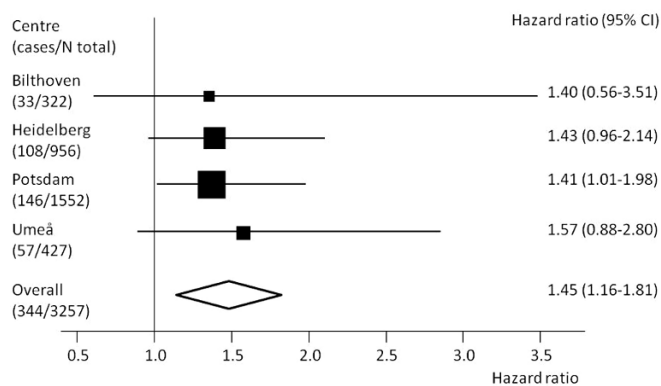


Figure 1. Forest plot showing adjusted hazard ratios and 95% CIs for the centres included in the study investigating the association between low self-rated health and mortality in patients with type 2 diabetes.

PS 088 New devices

1005

How accurate are blood glucose meters used for patient self testing

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Background and aims: There is increasing concern being expressed about the accuracy of glucose meters used both in hospitals and for self-testing. A number of endogenous and exogenous substances can influence the accuracy of results and as such several bodies (ISO, IFCC, FDA) are looking at revised performance criteria for glucose meter performance. The purpose of this study was to challenge the analytical performance of commonly used SMBG meters and to compare the clinical performance of the best and worst performing meters.

Materials and methods: Seven SBGM meters (NovaMax plus, Glucofix Mio plus, Glucomen Lx plus, AccuCheck Aviva, Ascencia Breeze 2, Optium Xceed, and OneTouch Ultra 2) were tested. The meters were challenged with differing hematocrit levels and differing levels of non-glucose sugars (maltose, galactose, xylose) and at five different glucose concentrations (1.1–3.3, 5.5–8.3, 11.1–16.7, 18.1–22.2 and 23.6–27.8 mmol/L). Each individual sample was tested 6 times with each meter. Results were compared to the YSI reference method and the mean bias deviation calculated for each meter. The imprecision of each meter was determined at three different levels and this was used in conjunction with the bias deviation to calculate mean total error (%bias + 1.65 CV(%)). A method correlation was performed using a spiked sample panel. The meters showing the best and poorest total errors were selected for a study performed on capillary samples collected from self-testing diabetic outpatients.

Results: An example of the mean total error rates for all glucose meters at a glucose range of 11.1–16.7 mmol/L is presented in the Table. The Nova Max plus, Glucofix mio and Glucomen Lx were unaffected by the interferences assessed and demonstrated low and acceptable total error rates. The other meters were affected to varying levels by the interfering substances. The Nova Max plus, Glucofix mio and Glucomen Lx and One Touch Ultra were selected for diabetic patient testing. Initial data shows that the varying levels of haematocrit seen in diabetic outpatients, also affect the accuracy of One Touch Ultra.

Conclusion: The accuracy of SMBG meters can be affected by haematocrit as well as non-glucose sugars. The results pattern seen in the analytical assessment for haematocrit also occurs with real patient samples. In accurate meters increase the risk of mis-management of diabetes and new performance criteria for SMBG meters need to take this into account.

Meter	Method correlation	Hct (22%)	HCT (62%)	Maltose (5.6 mmol/L)	Galactose (5.6 mmol/L)	Xylose (5.6 mmol/L)
NovaMax Plus	8.5%	9.3%	5.8%	6.8%	6.7%	7.8%
Glucofix mio Plus	7.8%	3.8%	5.8%	11.5%	9.1%	6.4%
Glucomen Lx Plus	7.2%	7.1%	4.1%	8.3%	10.4%	4.7%
AccuCheck Aviva	9.5%	20.2%	13.4%	20.2%	68.0%	15.4%
Ascencia Breeze 2	9.5%	31.6%	32.7%	19.8%	13.0%	9.3%
Optium Xceed	15.5%	28.9%	46.8%	14.7%	11.5%	13.6%
One Touch Ultra	16.6%	42.9%	43.0%	17.0%	21.9%	16.3%

Supported by: Nova Biomedical

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First clinical assessment of a blood glucose predictor and therapy advisor in type 1 diabetes: The DIAdvisor-1 Trial

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Background and aims: Timely adjustments of insulin doses in type 1 diabetes (T1D) according to variable insulin needs remain a heavy burden for patients in everyday life. In spite of specific education, the common failure in achieving this task results in frequent out-of-target blood glucose control. The

DIAdvisor device has been designed in view of predicting blood glucose at a close time horizon and delivering advice on correction measures in order to maintain blood glucose in a near-normal target range in T1D patients using a basal-bolus insulin regimen by multiple daily insulin injections or insulin pumps. Inputs include patient information on carbohydrate intakes, insulin dose administration and continuous glucose monitoring (CGM) data. In a pilot study, we investigated the ability of collecting inputs by the system, the accuracy of blood glucose prediction at a 20-min horizon and the coherence of treatment advices.

Materials and methods: Sixteen T1D patients volunteered to assess DIAdvisor device in a Clinical Research Centre setting in three successive steps. Blocks of 4 patients were included successively, allowing for system refinement, until success criteria were reached at each step. The primary endpoints and success criteria were: in step A, a percentage of data collection from CGM for 48 hours > 80%; in step B, an accuracy of blood glucose prediction at a 20-min horizon assessed by using Clarke error grid analysis of paired predicted glucose values and YSI glucose measurements for 48 hours with > 80% of paired points in A+B zones and <5% in E zone; in step C, a coherence of advices given by the system on glucose and insulin corrections compared to clinician's advices for 48 hours > 80%. The DIAdvisor device includes an Ultra Mobile (UM) PC with a patient interface and a USB connection to a CGM DexCom Seven-Plus receiver. Algorithms for glucose prediction and treatment advice are uploaded on the UMPC. Predictions and advices were blinded to the patient but wirelessly transmitted to a nearby clinician monitoring station.

Results: In step A, the success criteria were reached after the inclusion of 8 patients with 85.7% of CGM data collected for 48 hours. In step B, the accuracy of glucose prediction at 20 min was validated after the inclusion of 8 patients as shown by 90.9–100% of paired points in A+B zones and 0% in E zone in the second block of 4 patients. In step C, the coherence of system advices reached 96.5% in a series of 11 consecutive patients with 85.7% full coherence in terms of suggested insulin or glucose correction amounts.

Conclusion: This pilot study shows that the DIAdvisor system is able to provide successful prediction of blood glucose at a 20-min horizon and coherent advices for treatment correction in T1D patients in a standardized hospital environment. Further trials will assess the DIAdvisor system in less controlled conditions.

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1007

Use of an automated bolus calculator reduces fear of hypoglycaemia and improves confidence in dosage accuracy in type 1 diabetes mellitus patients treated with multiple daily insulin injections

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Background and aims: Intensive management of glycemia can significantly reduce the development/progression of diabetic complications. Achieving optimal glucose control often requires intensive insulin therapy. Many patients, however, are reluctant to intensify (or even follow) their prescribed insulin regimen because of fear of hypoglycemia; patients with type 1 diabetes (T1DM) experience an average of two episodes of symptomatic hypoglycemia each week and at least one episode of severe, disabling hypoglycemia, annually. We hypothesized that utilization of an automated bolus calculator (ABC) might reduce fear of hypoglycemia and encourage patients to achieve improved glycaemic control.

Methods and materials: We sent a 45-item questionnaire to 1,412 T1DM patients treated with multiple daily insulin injection therapy (MDI) at 270 hospitals and clinics throughout the United Kingdom and Republic of Ireland. The questionnaire was designed to assess their attitudes and behaviors regarding insulin therapy after use of an ABC (Accu-Chek[®] Expert system, Roche, Diagnostics, Indianapolis, USA). The hand-held device allows patients to test their blood glucose and obtain meal and correction bolus recommendations based upon their current blood glucose value, planned carbohydrate intake, and patient-specific therapy parameters stored in the device.

Results: 588 T1DM patients responded to the survey. Respondents were predominantly female (57.5%), age 0 to 70 years (31.0% age less than 18, 56.8% age 18–50, 12.2% age 51–70), with diabetes duration ranging from 0–2 years (18.5%) to greater than 15 years (39.6%). Patients had 4–12 weeks experience using the ABC prior to receiving the questionnaire. More than 95% of respondents indicated that they used multiple daily insulin injection (MDI) therapy with a long-acting analog. 76.7% of 562 respondents indicated

that they use the ABC to calculate insulin boluses for meals/snacks always (53.6%) or quite often (23.1%); 232 (41.5%) of 559 respondents stated that they used the ABC to calculate correction boluses always (15.0%) or quite often (26.5%). 478 (89.3%) of 535 responders indicated that the ABC made bolus calculation easy or very easy compared with manual calculation: 44.9% vs. 26.0% and 44.5% vs. 8.4%, respectively. 292 (52.0%) of 561 respondents indicated that their fear of hypoglycemia was improved (39.0%) or significantly improved (13.0%); 442 (78.8%) indicated that their confidence in the insulin dose calculation either improved (50.8%) or significantly improved (28.0%).

Conclusion: Although most patients indicated consistent use of mealtime boluses based on carbohydrate counting prior to using the ABC, the majority of patients surveyed felt that using the ABC was easier than manual bolus calculation, improved their confidence in the accuracy of their bolus dosage, and reduced their fear of hypoglycemia. Randomized trials are needed to confirm these perceptions, and to determine whether ABC use improves clinical outcomes.

Supported by: Roche Diagnostics Limited

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Clinical evaluation of a new technology for blood glucose monitoring: accuracy at hypoglycaemic glucose levels

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Background and aims: Monitoring for hypoglycemia is an essential component of glucose testing at home; however, most of the data found in published accuracy evaluations fall in the normoglycemic or hyperglycemic ranges. We studied the accuracy of OneTouch[®] Verio[™] Pro test strips, a new blood glucose monitoring technology, at hypoglycemic glucose levels (<70 mg/dL) using data collected in four clinical studies conducted at two clinical sites.

Materials and methods: In each clinical study, testing was performed by clinic staff using fingertip blood samples from subjects with diabetes. The study population included 414 subjects, and testing was conducted using nine different lots of test strips. With each subject, duplicate tests with each test-strip lot were performed with the monitoring system using blood from a single fingertip lancing. The blood glucose concentrations of samples were targeted to achieve the distribution specified in standard testing guidelines (ISO 15197:2003 standard). Plasma glucose reference values were obtained before and after testing with the blood monitoring system by using the YSI 2300 STAT Plus[™] Glucose Analyzer system. The number and percentage of results within ± 15 mg/dL and ± 10 mg/dL were calculated at blood glucose levels <70 mg/dL and <60 mg/dL.

Results: In total, 366 blood glucose results were evaluated at concentrations <70 mg/dL (range 32–69 mg/dL). In this glucose range, 100% (366 of 366) of OneTouch VerioPro results were within ± 15 mg/dL and 99.5% (364 of 366) were within ± 10 mg/dL of YSI reference values. At glucose concentrations of <60 mg/dL, 100% (174 of 174) of OneTouch VerioPro results were within ± 15 mg/dL and 100% (174 of 174) were within ± 10 mg/dL of YSI reference values.

Conclusions: Reliable detection of hypoglycemia requires accurate blood glucose monitoring. In this preliminary evaluation, the data from four clinical studies suggest that the new monitoring technology utilized in the OneTouch VerioPro test strips is capable of providing highly accurate results at hypoglycemic blood glucose levels. Further studies are needed to confirm the long-term performance of the system.

1009

Evaluation of a non-invasive method to determine blood glucose levels based on near-infrared spectroscopy coupled to a neural network system

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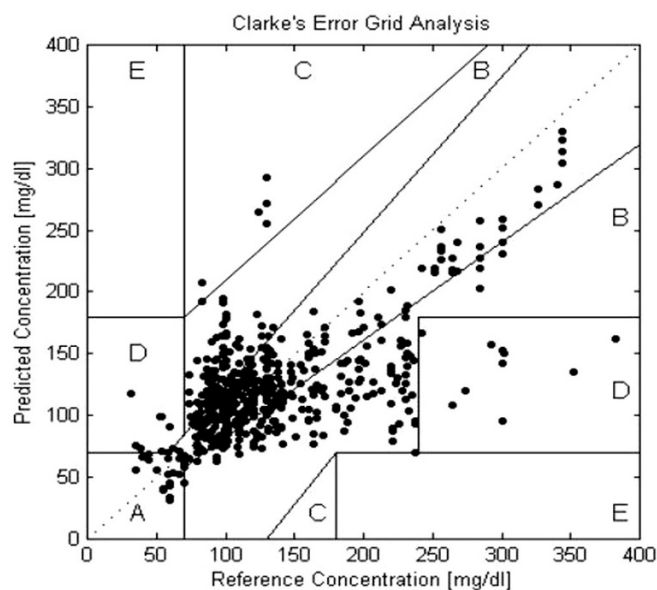
Background and aims: Tight control of blood glucose levels is essential for preventing or arresting diabetic complications. In those patients under insulin treatment, self-monitoring of blood glucose levels will be crucial for achieving the therapeutic objectives. The currently used devices use capillary blood obtained by pricking the fingers tips. Obviously, this procedure is annoying and for this reason a lot of diabetic patients reduce the number of auto-analyses to a minimum. Therefore, it is reasonable to postulate that

a non-invasive method that took the same time as the classic glucometers would improve the quality of life of diabetic patients and permit them to optimize their glycemic control, thus reducing both acute and late diabetic complications. The aim of this study was to assess whether a noninvasive method based on near-infrared spectroscopy to determine blood glucose levels had the same reliability as the meters currently used for blood glucose self-analysis.

Materials and methods: We prospectively evaluated 253 consecutive diabetic patients by comparing the estimated value of blood glucose using the new digital device with the venous glucose level determined simultaneously by the gold standard method (glucose-oxidase). The digital device is similar to a pulse oximeter, emits near-infrared (NIR) light and is coupled with a neural network system. In order to infer the blood glucose value, the prediction model included the following variables: age, sex, body mass index (BMI), saturation of oxygen (SaO₂), heart rate and blood pressure (the three last variables were determined at the time of blood collection). Data were analyzed by logistic regression analysis and the results were represented in the Clarke Error Grid.

Results: As can be seen in the figure, 95.5% of the blood glucose values estimated by the noninvasive method were located in areas with high accuracy of the Clarke's error grid (A and B zones). Only in patients with morbid obesity and severe hypertension we detected errors that are not acceptable for the current clinical practice (C, D, and E zones of the Clarke Error Grid).

Conclusion: The proposed noninvasive test is reliable for measuring blood glucose levels and could replace current blood glucose meters. The main consequences for the health care systems would be the following: 1) Improvement of the quality of life of diabetic patients. 2) Increase of self-monitoring, thus resulting in better metabolic control. 3) Reduction in the economic burden related to diabetes due to the dramatic decrease in glucose test strips consumption.



1010

Progressing towards a truly non-invasive glucose monitor for home use
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Background and aims: Non-Invasive (NI) tracking of physiological phenomena correlated with Blood Glucose (BG), using a single method encountered obstacles of non-specificity, since factors, other than glucose, influence tissue parameters as well and cause inaccuracies in the reading. In order to minimize the impact of those disturbances, a methodology combining multi-technology and multi-sensors was previously introduced. Each technology measures different tissue parameters that are affected by the same change in glucose, but is confined by the impact of interfering factors, due to lack of specificity. Therefore, a simultaneous evaluation of the above mentioned

physiological changes through measurement of different sets of tissue perturbations, induced by changes in glucose concentration, is expected to increase the validity of the end result. GlucoTrack[®] glucose monitor combines 3 NI technologies: Ultrasonic, Electromagnetic and Thermal. These technologies, together with the adjunct sensors, are located at the tip of a Personal Ear Clip (PEC), which is clipped externally (non-invasively) to the earlobe for the duration of less than a minute, to conduct a spot measurement and is removed afterwards. The weighted average reading reflects the BG value with smaller impact of interferences, leading to more accurate real-time, spot readings.

Materials and methods: The device performances were evaluated in Clinical trials, initially launched in a clinic and sequentially followed by home trials. In the Clinic group, 91 subjects were evaluated (1772 data pairs): 12 T1DM and 79 T2DM, 41 F and 50 M, age 51.0 ± 30.0 years and BMI of 30.0 ± 10.0 Kg/m². The trial was performed in two different days per each individual. The time intervals between calibration and measurement days were 11.5 ± 10.5 days. In the Clinic group, GlucoTrack and the invasive reference device related actions (calibration and measurements) were performed in the clinic by a proficient medical staff. For evaluation of the upgraded device performances and ease of use in home environment, 12 subjects were tested (800 data pairs): 2 T1DM and 10 T2DM, 4 F and 8 M, age 47 ± 22 years with BMI of 31.7 ± 5.0 Kg/m². The calibration was individually performed by the medical team. Post-calibration measurement procedure was performed by the subjects themselves in their home/work environment for 2-37 days, with a median of 16 days.

Results: Clarke Error Grid (CEG) analysis for the in-clinic group shows 97% of the points in the clinically accepted zones A+B, of which 60% in zone A. MARD_{mean} is 22.4 % and MARD_{median} is 16.3 %. CEG analysis for the Home group shows 94% of the points in the clinically accepted A and B zones, of which 54% in zone A. MARD_{mean} is 25.3 % and MARD_{median} is 18.3%.

Conclusion: The presented methodology shows promising results in both, Clinic and Home groups. The initial home trials show no significant degradation relative to the results obtained in the clinic, including relatively long time after calibration. However, when comparing between home and clinic groups' results, small variation is observed. The small deviations observed might be attributed to user related issues, e.g. disobeying the rules of correct invasive measurement. The key benefits of GlucoTrack include long intervals between re-calibrations and the ability to perform frequent spot measurements with acceptable accuracy, present high likelihood for a solution to improve BG monitoring adherence.

Clinical Trial Registration Number: 4823

1011

Evaluation of performances and efficacy of GlucoTrack[®], a truly non-invasive glucose monitor for home and home-alike use

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Background and aims: Inconvenience, expenses, pain and complexity involved in conventional SMBG, lead to under-utilization. Availability of an accurate, painless, easy to operate and non-expansive device will encourage more frequent testing. Previous publications indicate that GlucoTrack[®], a Non-Invasive (NI) glucose monitor, is able to measure glucose by using a combination of three technologies: Ultrasonic, Electromagnetic and Thermal. GlucoTrack comprises a Main Unit and 3 different sensor pairs, assembled at the tip of a Personal Ear Clip (PEC). The measured tissue parameters reflect physiological changes occurring in the tissue, correlated with blood glucose variation. Interpretation of these parameters into a glucose value is done based on individual calibration process. The calibration minimizes the affect of the individual quasi-stable factors such as tissue thickness and structure, sets a baseline for physiological change detection and allows finding optimal glucose behavior model parameters to be suited for each user. The multi-technologies combination is essential to reduce inaccuracies in the glucose readings, since factors, other than glucose, influence the measured tissue parameters as well. Thus, the weighted average reading of the three technologies reflects blood glucose value with smaller impact of interferences, leading to more accurate, real-time spot glucose readings.

Materials and methods: GlucoTrack performance was evaluated in Home use environment for 12 subjects: 2 T1DM and 10 T2DM, age 47 ± 22 years with BMI of 31.7 ± 5.0 Kg/m². The calibration was individually performed by a medical team, using invasive blood glucose references. This easy procedure takes about 1.5-2 hours and is valid for at least over a month. Post-calibration spot glucose measurements were performed by clipping the PEC externally

to the earlobe. Each subject performed the measurement procedure by him/herself at home/work environment for 2–37 days, with a median of 16 days. Data records were collected according to participants' own routine of glucose monitoring (timing and number of readings per day), but no less than 5 measurements per day. During each measurement, simultaneous pairs of GlucoTrack and invasive reference readings were collected. GlucoTrack readings were compared with the participants' own (invasive) glucose monitoring devices, which served as the reference for calibration, as well as for comparison of measurements. In addition, users' feedbacks regarding operation were analyzed.

Results: Clarke Error Grid analysis of 800 data points shows 95% of the readings within the clinically accepted A and B zones, of which 54% in zone A. $MARD_{mean}$ is 25.3% and $MARD_{median}$ is 18.3%. 97% of the users found the device to be comfortable for use and 87% of the users declared they'll use the device more frequently than the invasive one. Home trials are still currently ongoing.

Conclusion: Initial readings under real home conditions suggest that GlucoTrack gives good results. The users' feedbacks indicate that the device is user friendly and easy to operate under normal home environment and conditions, as well as willingness to use it more frequently. Such a device can be beneficial for diabetics in improving monitoring adherence and allow diabetics a frequent and painless way to monitor and track their BG, leading to tighter glucose control.

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Evaluating glucose area under the curve (AUC) measurements by minimally invasive interstitial fluid extraction technology: comparison to AUC measured by continuous glucose monitor

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Background and aims: Controlling postprandial glycemic excursions is important. However, monitoring blood glucose 2 hours after a meal by self-monitoring of blood glucose is not always appropriate since the glycemic excursions depend on several factors such as dietary content and patient's condition. We have newly developed the monitoring system for glucose area under the curve (AUC) using minimally invasive interstitial fluid extraction technology (MIET), which enables us to perceive the complete glucose excursion as the area measured. We have confirmed that glucose AUC measured using MIET correlated well with that calculated with plasma glucose during OGTTs in diabetic patients. In this study, we compared glucose AUCs measured using MIET with those measured by continuous glucose monitoring (CGM).

Materials and methods: Thirteen diabetic patients underwent CGM (CGMS-Gold, Medtronic) and were simultaneously measured glucose AUC using MIET. MIET consists of the following two steps. First, a plastic micro-needle array is applied to the skin of the forearm as the pretreatment to enhance transdermal interstitial fluid glucose (IG) extraction. It does not cause any pain or bleeding, since the length of each microneedle is only 0.3 mm. Second, a hydrogel patch is placed on the pretreated area to accumulate IG for a predefined period. Hydrogel patches were attached at two sites pretreated with microneedle arrays, and replaced by new patches every 2 hours for 6–8 hours. Patients had breakfast and lunch during this period. After the extraction of IG, accumulated glucose levels in the hydrogel patches were measured to predict IG-AUC. Extracted sodium ion levels were simultaneously measured for the calibration.

Results: Predicted AUC using MIET correlated well with that using CGM ($r = 0.84$) over a wide AUC range (170–548 mg·h/dl). Correlation level was independent of the IG level, IG variation rate, IG variation range, or extraction period. The mean CV of the two simultaneous measurements at different sites was 6.3%. The patient questionnaire confirmed that this method can be done without any pain.

Conclusion: We confirmed that extracted IG using MIET correlated well with the IG measured by CGM. Our convenient and painless monitoring system without blood sampling could be an excellent tool for a better understanding and management of postprandial glycemic control.

Supported by: Sysmex

PS 089 Socio-economic aspects

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The clinical and economic burden of type 1 diabetes in children, adolescents and adults

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Background and aims: Type 1 diabetes (T1D) represents approximately 10% of the global prevalence of diabetes and imposes a substantial burden on the health care system. We measured the clinical and economic burden of T1D as a necessary platform from which to plan effective health care strategies. The objectives were to estimate: 1) the direct medical costs of T1D; and 2) the proportion of subjects achieving glycaemic control, among children (8 to 12 years), adolescents (13 to 18 years) and adults (19 to 35 years).

Materials and methods: We designed a retrospective population-based cohort study of insulin-dependent subjects using clinical and administrative data held in the Health Informatics Centre, Tayside, Scotland (2000–2008). Subjects who received insulin for less than nine months or an oral antidiabetic prior to initiating insulin were excluded. We estimated the mean annual direct medical cost of T1D-related resource utilization (diabetes clinic visits, eye and foot screening, hospitalizations, laboratory tests, insulin, and glucose testing strips). Sub-optimal glycaemic control was defined as HbA1c > 7.5% for children and adolescents and HbA1c > 7% for adults. Poor glycaemic control was defined as HbA1c > 9.5%. Descriptive statistics were calculated by year and stratified by age.

Results: We identified a mean of 527 subjects per year with prevalent or incident T1D. The mean period prevalence per 1,000 person years was: 2.56 (children); 3.77 (adolescents); and 3.86 (adults). The mean incidence rate per 100,000 person years was: 49.6 (children); 44.5 (adolescents); and 27.0 (adults). Between 87% (adults) and 95% (adolescents) of subjects had at least one annual visit to a diabetes clinic. Most adults received annual eye and foot screening (78% and 79%, respectively); however, fewer than 5% of children used these services. The mean length of stay per hospitalization was 6.7 days for children (SD 2.2), 19.0 days (SD 8.6) for adolescents and 25.6 days (SD 16.5) for adults. Ketoacidosis was the most common reason for hospitalization, with 5.5% (adults) to 9.3% (adolescents) of individuals hospitalized annually. Between 52% (children) and 76% (adults) of subjects received annual micro-albuminuria tests, and 38% (adults) to 42% (children) of subjects received thyroid tests. The mean annual direct medical cost per person (in 2009 Great Britain Pounds) was: £2,747 (95% CI: £2,154 to £2,668) among children; £6,655 (95% CI: £5,660 to £7,170) among adolescents; and £4,023 (95% CI: £3,828 to £4,155) among adults. The largest cost driver was hospitalizations. Costs for blood glucose testing strips appeared to increase over the period. Most subjects had at least one HbA1c test per year (87% (adults) to 95% (adolescents)); however, few achieved target levels (between 10% (adolescents) and 32% (adults)) and many had poor glycaemic control (between 23% (children) and 46% (adolescents)).

Conclusion: This study provides a unique assessment of the burden of T1D among children, adolescents and adults. In the last decade, a more intensive approach to T1D management has been adopted, reflected in the rising use of blood glucose strips; however, hospital admissions remain a problem, and glycaemic control is not optimal in the majority of these subjects, particularly adolescents. Despite the current costs, newer technological therapies must be considered to achieve long-term improvement in the health of young people with T1D.

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Burden of disease, cost and management of diabetes in EU5 countries

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Background and aims: Diabetes mellitus is associated with high risk of developing complications & severe comorbidities. In recent years the prevalence of Type I & II diabetes and its associated costs (esp. those related to treatment of complications and management of the disease) have risen. However, EU5 (France, Germany, Italy Spain and the UK) governments are not fully aware of the economic, health and societal implications of diabetes. The aims of this research are to identify and compare the evidence on the burden of disease,

costs (both direct and indirect), outcomes of diabetes across EU5 countries and to link costs and outcomes to the extent this is possible. It also aims to provide recommendations to policymakers.

Materials and methods: Primary data were collected through a questionnaire survey completed by researchers and clinicians, combined with structured interviews with clinicians and decision makers in the study countries; secondary data were collected from international databases, the international peer review literature and national statistics.

Results: The diabetes burden of disease is high and growing with an estimated country prevalence of between 3.6% (UK) and 8.9% (Germany). The direct costs of diabetes treatment are substantial, ranging from €5.1bn (Spain) to €42bn (Germany) per year, in total. Per patient direct costs are more comparable across countries, with some variation (€1,660 (Spain) to €5,726 (Germany)), indicating that the key driver behind total diabetes expenditure is prevalence. The total direct cost burden in the 5 study countries stands at €80 billion. In-patient care costs represent the highest component of direct costs (33–49%), followed by out-patient care (18–36%) and pharmaceuticals (including diabetes and non-diabetes drugs (20–32%). The cost of diabetes-related drugs (insulins and oral anti-diabetics) are a fraction of total costs and do not exceed 7% of the total. A significant majority of the in-patient direct costs accounts for treatment of diabetes-related complications, affecting approximately 18.3 million diabetic patients each year across the five study countries. Indirect costs, quantified in EU5 for the first time, relating to reduced productivity, absenteeism, early retirement and disability, are significant and can exceed direct costs by a factor of 2 to 1. Significant variations exist in the availability of outcomes data between countries and the quality of the relevant indicators. In some cases improvements in quality of care for diabetic patients are shown over time (Italy), whereas in others substantial discrepancies exist between the quality of care in metropolitan areas compared to rural settings (France).

Conclusion: Rising prevalence and costs of diabetes are growing concerns. Also of concern is the lack of adequate and accurate prevalence, cost estimates, particularly the indirect, and outcomes data. Therefore the true extent of the problem that diabetes poses is likely highly underestimated. Monitoring and evaluation of diabetic patients are also areas requiring attention. Diabetes poses a serious problem to all study countries in terms of its economic costs; the burden on individuals, families, national health systems, labour markets and national economies is considerable and will only increase in the future. Policymakers need to act urgently and improve funding and prioritisation of this disease and to drive enhanced preventative initiatives and co-ordinated disease management if we are ever to manage its total cost and societal impact.

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Cost-effectiveness analysis of medical intervention in patients with early detection of diabetic neuropathy in a tertiary care hospital in Bangladesh

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Background and aims: The economic burden resulting from diabetic neuropathy (DN) consumes a major portion of resources allocated for health-care services. Cost-effectiveness of various interventions on DN and its complications has relatively been well explored in developed countries, but these are almost absent in developing countries. The present study was undertaken to assess the cost-effectiveness of medical intervention in patients with DN.

Materials and methods: Two hundred patients with DN, at least 1 year of follow-up, were purposively selected from Out-Patient Department of BIRDEM (tertiary diabetes care hospital), Bangladesh. Of them 100 were late in detection of DN (LDN) and 100 were detected early (EDN). The degree and extent of complications like cardiopathy, retinopathy, nephropathy and vasculopathy, treatment outcome, clinical effectiveness of interventions and direct, indirect & incremental cost of complications were calculated. Comparison was made between the groups.

Results: A total of 200 patients were considered for an average of 365 days, amounting to 656 person-years of observation in total. In LDN group, 22% had Diabetic Peripheral Neuropathy (DPN), 17% had Diabetic Autonomic Neuropathy (DAN), 11% had Diabetic Proximal Neuropathy (DPXN) & 9% had Diabetic Focal Neuropathy (DFN). In EDN group, 16% had DPN and 7% had DAN. The mean±SD fasting serum glucose of the groups (LDN & EDN respectively) was 10.1±0.4 & 6.1±0.3 mmol/l, TG was 163.7±99.4 & 155.6±94.8 mg/dl, total cholesterol was 205.5±41.6 & 103.2±34.5 mg/dl, HDL cholesterol was 56.2±20.3 & 39.0±14.1 mg/dl, HbA_{1c} was 9.2±1.5% &

6.5±1.3%, SBP was 172.5 ± 20.9 & 109.5±11.9 mmHg and DBP was 97.7±10.0 & 70.7±9.3 mmHg. About 19% patients in LDN & 36% in EDN were free of diabetic complications other than DN. In LDN & EDN, 32% and 48% had one complication, 29% and 10% had two and 20% and 6% had more than two complications respectively. The most frequent complication was cardiopathy, which affected 33% patients in LDN and 27% in EDN, followed by retinopathy 21% and 18%, nephropathy 17% and 13%, and vasculopathy 10% and 6% respectively. The average annual cost of care was US\$ 26846 (direct US\$ 17893 & indirect US\$ 8953), with an average US\$ 134 per patient. Among the average annual cost LDN consumed US\$ 18918 (US\$ 189 per patient) & EDN US\$ 7928 (US\$ 79 per patient). US\$ 13473 (50%) of costs was attributable to Drugs for both groups of which US\$ 10419 (77%) was for LDN & US\$ 3054 (23%) for EDN, US\$ 7653 (29%) to hospitalizations of which US\$ 4914 (64%) for LDN & 2739 (36%) for EDN. In case of diagnostics and visits the corresponding values were US\$ 1953 (55%) and 1580 (45%) and US\$ 1631 (75%) and 556 (25%) for LDN and EDN respectively. The annual medical costs increased with the increased number of complications from US\$ 1320 to 2296 to 3989 and to 6520 in LDN with one, two, three and more than three complications (other than DN) which is increasing at a rapid rate and US\$ 917 to 1556 to 1872 and to 2073 in EDN respectively, increasing at a diminishing marginal rate. The regression equation showed that medical cost is significantly related to complications tested in both univariate ($P < 0.0001$) & multiple linear regression analyses ($R^2 = 0.69$; $F = 81.5$, $P < 0.0001$).

Conclusion: Proper management with regular screening substantially reduces the expenditure related to care and complications of patients with DN even in a developing country. Strategies aimed at preventing DN and early detection of the onset of neuropathy will reduce medical costs in a substantial way.

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Direct medical cost of diabetes mellitus in Albania: a first study in hospitalised and out-patients polyclinic

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Background and aims: The burden of Diabetes Mellitus is increasing rapidly worldwide. The continuous prevalence raise of Diabetes is another factor influencing the share of diabetes cost in the health expenses of developed and developing countries. The aim of our study is to determine the direct cost per patient and the overall cost of diabetes mellitus in a group of Albanian diabetic adults.

Materials and methods: A retrospective, observational study, based on clinical audit through the analysis of the direct cost for hospitalized patients and a group of out-patients, followed in a polyclinic in Tirana. Cost was expressed only as direct means (drugs, laboratory tests, doctors, hospitals, health care). Data used for these calculations come from two sources: 1). From the individual medical records of the diabetic patients of out-patient polyclinic Nr.8 in Tirana (total of 92 patients), covering the period from January to November 2010. 2) The medical records of admitted patients at the Service of Endocrinology, in our hospital in Tirana, for the period May-June 2010 (total of 190 patients).

Results: The direct recovery cost for a patient was 146.54€, for an average hospitalization of 7.11 days (20.61€/day). As for indirect costs or social point of view there is no data or studies available in Albania. For the out-patients, the cost of diabetes treatment and follow-up was 13.43€ per month, while the cost for diabetes complications was 34.8€ per month.

Conclusion: Our study is the first one trying to estimate the direct annual cost of diabetes in Albania. We estimated the direct cost for hospitalized and out-patients with diabetes. Diabetes complications (acute and chronic) increase two to three fold the cost of Diabetes treatment. The diabetes' economic burden is significant and it's comparable to results from other countries. For indirect costs or social point of view there is no data or studies available.

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Costs and clinical outcomes after 24 months of insulin therapy in patients with type 2 diabetes: results from the TREAT study

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Background and aims: Insulin treatment remains a challenge in daily clinical practice. TREAT was a prospective 24-month observational study in patients (pts) with type 2 diabetes (T2D) initiating insulin, in a routine clinical setting, in 5 countries. The objectives of the study were to assess the costs associated with insulin treatment, describe starter insulin regimens and changes over 2 years as well as metabolic outcomes.

Materials and methods: Data on disease, patient characteristics, and medications were collected at baseline, and medications, clinical, and economic outcomes at 3, 6, 12, 18 and 24 months after insulin initiation. The association of patient and disease characteristics with change of therapy was assessed using logistic regression.

Results: A total of 985 pts were enrolled: Greece (GR, 205), Portugal (PT, 165), Romania (RO, 207), Sweden (SE, 178), and Turkey (TR, 230). Retention was good, with data available for 766 pts at baseline and 755, 725, 696, 658, 629 at 3, 6, 12, 18 and 24 months, respectively. Mean age at baseline was 60.6 years and time since diagnosis, 10.2 years. Mean (SD) total cost of T2D care over 24 months was: 2642€ (1054) for GR, 2630€ (1664) for PT, 1169€ (446) for RO, 3142€ (2284) for SE and 1230€ (499) for TR. At baseline, 49% of pts started with a long/intermediate acting (Long) insulin, 41% with mixtures (Mix), 7% with basal-bolus (Bas-Bol), and 2% short-acting only. The percentages for each respective regimen by country were GR (65%, 29%, 4%, 1%), PT (56%, 43%, 1%, 0%), RO (44%, 42%, 7%, 6%), SE (59%, 35%, 5%, 1%), TR (28%, 53%, 16%, 2%). There were few changes in insulin regimens over 24 months, with 81% of patients having no other insulin prescribed. Initial treatment with Long insulin was positively associated with prescription of additional insulins ($p=0.004$). Blood glucose monitoring was performed in 78% of pts at baseline and 94% at 24 months. The percentage of pts reporting hypoglycemic episodes was approximately 20% at all visits with about 90% resolved without assistance. Evolution of HbA1c is described in Table 1. At 24 months, 33.9% of pts achieved an HbA1c $\leq 7.0\%$ and 54.0% $\leq 7.5\%$. Mean total daily insulin dose was 26.0 U (0.32 U/kg) at baseline and increased to 38.9 U (0.46 U/kg) at 24 months. Mean weight was 82.4 kg at baseline and 85.6 kg at 24 months. Similarly, BMI increased from 29.9 to 31.1 kg/m².

Conclusion: The total mean cost varied among countries, ranging from 1169€ for RO to 3141€ for SE. HbA1c at baseline was relatively high, indicating late initiation of insulin. Different insulin regimens were used at initiation and varied across countries. There was a significant lowering of mean HbA1c over time observed in all initiation regimens.

Table 1: Evolution of HbA1c.

Mean HbA1c	All patients	Long	Mix	Bas-Bol	GR	PT	RO	SE	TR
Baseline	9.5%	9.0%	10.1%	10.2%	8.9%	9.6%	9.8%	7.9%	10.7%
24 months	7.5%	7.5%	7.5%	7.3%	7.2%	7.9%	8.0%	6.6%	7.6%

Supported by: Eli Lilly and Company

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Direct costs of care and clinical outcomes in patients with type 2 diabetes switching between short-acting human insulin and rapid-acting insulin analogues

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Background and aims: As compared to human insulin, rapid acting insulin analogues reduce postprandial hyperglycemia, reduce the injection-mealtime interval, reduce severe hypoglycemia and may decrease HbA1c to a greater extent. However, insulin analogues are more expensive than human insulins.

The SWING study assessed direct treatment costs, glucose control, satisfaction and quality of life in patients following switches from short-acting human insulins to rapid-acting insulin analogues (H-A) or vice versa (A-H).

Materials and methods: SWING was an observational, prospective, multicentre, 12-month study performed in 9 European countries (Austria, Czech Republic, Germany, Greece, Poland, Romania, Slovakia and Turkey). Adults with type 2 diabetes (T2D) were invited to participate when they were switching insulin type (H-A or A-H) in the course of normal clinical care. Data were collected at a baseline visit (switch) and at approximately 3, 6 and 12 months post-switch. Analysis was mainly descriptive.

Results: 2389 patients switched H-A (n=2203) or A-H (n=186) insulins (another 603 enrolled but without eligible switch). Mean (SD) baseline data (H-A/A-H): age 60.9(11.1)/59.5(11.0) yrs, T2D duration 12.7(8.3)/11.4(7.2) yrs, HbA1c 8.6(1.6)/8.9(1.6)%, BMI 30.6(5.2)/30.9(5.5) kg/m². Overall, 2103 (88%) of the patients were taking at least 1 concomitant medication. The most frequently cited reason for the switch was 'lack of effect' (74.8% H-A, 62.9% A-H). Mean (SD) direct diabetes-related costs (standardised to account to variable visit schedules) were €548.7 (865.8) 6 months prior to switch, €625.6 (1474.9) at 0-6 months and €568.6 (590.7) 6-12 months following switch for H-A, and €544.5 (421.0), €481.0 (301.5) and €461.6 (335.0) for A-H, respectively. Mean direct cost increases were observed in H-A cohorts at 0-6 months while decreases were observed in A-H cohorts in most countries. Absolute standardised values differed between countries (eg mean [SD] 0-6 months highest in Germany [n=200] €1605.6 [4455.0], and lowest in Turkey [n=81] €343.7 [145.6]). Mean (SD) HbA1c decreased over 12 months: -1.08% unit (1.53% unit) H-A, -1.17% unit (1.45% unit) A-H. Proportion with an HbA1c of $\leq 7\%$ at 12 months was 34.8% H-A, and 30.4% A-H. No clinically important changes in mean BMI, blood pressure or triglycerides. The reported incidence of hypoglycaemia (recalled over the preceding 6 months) decreased from baseline (40.1% H-A, 39.8% A-H) to 12 months post-switch (28.5% H-A, 28.6% A-H), as did the mean (SD) number of episodes among those who did have hypoglycaemia (7.7[8.7]-3.6[4.1] H-A, 7.6[7.5]-5.5[4.3] A-H). There were no clinically meaningful changes from baseline in mean quality of life or treatment satisfaction questionnaire scores.

Conclusion: There were only small changes in mean direct diabetes-related costs (following adjustment for time interval) or patient-reported outcomes in patients switching in either direction between rapid-acting insulin analogues and short-acting human insulin. However, mean HbA1c and incidence of hypoglycaemia did improve following switch. The extent to which this observation can be attributed to therapeutic switch is unclear.

Supported by: Eli Lilly and Company

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Clinical and economic outcomes in patients with type 2 diabetes initiating insulin glargine using disposable pen vs exenatide

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Background and aims: Initiation and acceptance of insulin therapy for type 2 diabetes mellitus (T2DM) is limited, partly due to perceived complexity that may reduce adherence and increase risk of hypoglycemia. Insulin pens may help enhance patient acceptance of insulin therapy and facilitate diabetes management. Evaluate the clinical and economic outcomes in patients with T2DM who failed oral antidiabetic drug (OAD) therapy and initiated either insulin glargine with disposable pen (GLAR Pen) or exenatide (EXE Pen).

Materials and methods: This retrospective study used data from a large US managed care claims database and included T2DM patients initiating treatment with the GLAR Pen or EXE Pen between 2006 and 2009 who were aged ≥ 18 , treated with an OAD only with hemoglobin A1c (HbA_{1c}) $> 7\%$ and continuously enrolled in the health plan ≥ 6 months before and 12 months after initiation. Propensity score matching with baseline sex, age, region, health plan, comorbidity, HbA_{1c}, treatment, health care utilization and cost removed observed baseline treatment-group differences. Primary study endpoints included treatment persistence, HbA_{1c}, health care utilization, and cost during the 1-year follow-up period and were compared between treatment groups.

Results: A total of 2339 patients were included in the study (GLAR Pen: 381; EXE Pen: 1958); 626 patients were in the 1:1 matched cohort with no significant differences in baseline characteristics between groups (54% male; mean age 54 years; HbA_{1c} 9.2%). At the end of 1-year follow-up, patients in the GLAR Pen group were significantly more persistent in treatment compared with the EXE Pen group (48% vs 15% in persistence rate and 252 vs 144 days in persistent days, both $p<0.001$, respectively). They also had sig-

nificantly lower HbA_{1c} at the end of follow-up (8.02% vs 8.32%, $p=0.042$) and higher HbA_{1c} reduction from baseline (-1.23% vs -0.92%, $P=0.038$). There were no significant differences in hypoglycemia rates and overall diabetes-related health care utilization and cost. Compared with the EXE Pen group, patients using the GLAR Pen had significantly higher diabetes-related outpatient (\$1673 vs \$1473, $P=0.033$) and diabetes supply (\$282 vs \$161, $P<0.001$) costs, although these were offset by significantly lower diabetes pharmacy cost (\$2106 vs \$2438, $p=0.001$).

Conclusion: This study suggests that in the real world setting among T2DM patients who failed OADs, initiation of GLAR Pen instead of EXE Pen may be associated with greater persistence and improved clinical outcomes without increased hypoglycemia or cost. These findings need to be confirmed in further clinical studies.

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Real-world clinical and economic outcomes in patients with type 2 diabetes initiating glargine disposable pen vs. insulin detemir disposable pen in a US managed care setting

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Background and aims: Real-world data comparing outcomes of patients with type 2 diabetes mellitus (T2DM) initiating different insulin regimens would assist with treatment decisions and help optimize the management of T2DM. We compared clinical outcomes and healthcare costs following initiation of insulin glargine disposable pen (GLA-P) or insulin detemir disposable pen (DET-P) in T2DM patients.

Materials and methods: A retrospective cohort study was conducted using IMPACT, a US national managed care claims database (Jul 2006-Sep 2010). Patients were included if they were diagnosed with T2DM, aged 18-79 yrs, had HbA_{1c} data at baseline and end of 1-yr follow-up, were on ≥ 1 oral antidiabetics (OAD) or glucagon-like peptide-1 (GLP-1) medication but no insulin before initiation of GLA-P or DET-P. Patients had to be continuously enrolled in a health plan for 6-month pre- (baseline) and 12-month post GLA-P or DET-P initiation (follow-up). 1:1 propensity score matching was applied to patients in each cohort using their baseline demographic, comorbidity, HbA_{1c}, OAD or GLP-1 medication use, healthcare utilization and cost data (table). Treatment persistence and adherence, HbA_{1c} at the end of 1-yr follow-up, hypoglycemia events, and healthcare costs during the 1-yr follow-up were compared between the cohorts.

Results: A total of 1,682 patients were identified, 1016 (60.4%) initiating GLA-P and 666 (39.6%) initiating DET-P. After propensity score matching, each cohort contained 640 patients and they were well balanced (mean age 55.0 vs. 54.8 yrs; male 55.9 vs. 58.0%; CCI 0.6 vs. 0.6, HbA_{1c} 9.4 vs. 9.4%; all $P>0.4$). During follow-up, patients initiating GLA-P were significantly more likely to be persistent and adherent with treatment vs. those initiating DET-P (table). The average daily consumption (DAICON) dose was lower in the GLA-P group. Over the last quarter of the 1-yr follow-up, fewer GLA-P patients switched to DET-P vs. those switching from DET-P to GLA-P (1.4 vs. 5.9%). At the end of 1-yr follow-up, initiation of GLA-P was associated with lower HbA_{1c} and higher reduction of HbA_{1c} from baseline, without a significant difference in the number of patients having hypoglycemia events during follow-up (table). Patients in the GLA-P and DET-P cohorts had similar total and DM-related healthcare costs (table). Population-wise, for each 1% reduction in HbA_{1c} from baseline to the end of follow-up, the mean DAICON and healthcare costs were lower in the GLA-P vs. the DET-P group (DAICON: 23.6 vs. 31.8 units, diabetes-related cost: \$5,732 vs. \$6,287, total cost: \$13,059 vs. \$15,350, respectively).

Conclusion: This real-world study demonstrated that patients initiating GLA-P were more likely to persist and adhere with treatment, with better glycemic control. Furthermore, the initiation of GLA-P was more cost effective compared with DET-P.

Follow-up of the matched cohorts	GLA-P (n=640)	DET-P (n=640)	P-value
Persistence, n (%)	263 (41.1)	200 (31.3)	0.0002
Persistence days, days \pm SD	236.3 \pm 118.8	212.8 \pm 114.9	0.0003
Adherence measured by Adjusted MPR, mean \pm SD	0.7 \pm 0.3	0.6 \pm 0.3	0.0001
Adherence measured by Adjusted MPR ≥ 0.8 , n (%)	309 (48.3)	266 (41.6)	0.0157
DAICON, mean \pm SD	29.0 \pm 19.1	31.8 \pm 23.3	0.0342
HbA _{1c} at the end of 1-year follow-up, mean \pm SD	8.1 \pm 1.6	8.4 \pm 1.8	0.0260
Change in HbA _{1c} from Baseline, mean \pm SD	-1.23 \pm 2.09	-1.00 \pm 2.03	0.0467
Patients with Hypoglycaemia events, n (%)	49 (7.66)	36 (5.63)	0.1445
Total healthcare costs (US\$), mean \pm SD	16,063 \pm 20,270	15,350 \pm 20,291	0.5293
Diabetes-related costs (US\$), mean \pm SD	7,050 \pm 12,076	6,287 \pm 6,910	0.1656

Medication persistence was defined as the % of patients remaining on therapy without discontinuation or switching during the 1-yr follow-up.

Medication adherence was measured using the medication possession ratio (MPR) adjusted for differences in insulin device package size during the 1-yr follow-up.

Hypoglycemia was defined as a health care encounter with a diagnosis code for hypoglycemia (ICD-9 code 250.8; 251.0; 251.1; or 251.2) during the 12-month follow-up period.

GLA-P, insulin glargine disposable pen; DET-P, insulin detemir disposable pen, MPR, medication possession ratio, DAICON, daily average consumption.

Supported by: sanofi-aventis US

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In the US-setting, blood glucose testing represents 20% of total pharmacy diabetes-related costs in patients with diabetes on an insulin regimen

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Background and aims: For patients with insulin-requiring diabetes, Self-Measured Blood Glucose (SMBG) is an essential part of titrating and monitoring an insulin-based treatment regimen. However, little is known about the real-life frequency and costs associated with SMBG in relation to the specific regimen, and what SMBG expenditure is compared to other treatment costs.

Materials and methods: The present study used the IMS LifeLink Health Plan Claims Database, which have medical and pharmacy claims for more than 71 million unique patients from 102 health plans across the US. Patients were included in the analysis if they: had 2+ prescriptions for insulin (any type) during the period from January 1, 2007 through June 30, 2009; had 18+ months of data (6 months for baseline data + 12 months for outcomes data); had a diagnosis for type 1 or type 2 diabetes; were persistent with insulin therapy throughout the 12-month follow-up period. Patients were excluded if: they were <4 years old; they had incomplete claims data; their insulin type could not be adequately categorised.

Results: A total of 74,936 patients met the inclusion and exclusion criteria. All analyses were stratified by gender, age group, type of diabetes, insulin regimen (basal only, premixed, basal-bolus), type of health plan/payer, geographic region, total healthcare costs for all conditions during the patient's 6-month baseline period, Charlson Comorbidity Index, daily average consumption of insulin. The analysis demonstrated that SMBG (strips, lancets etc.) constitutes 20.2% (\$602 of \$2,975) of the total annual diabetes-related pharmacy costs (strips, insulin, needles, oral antidiabetic drugs etc.). Relative cost of SMBG was 15.3% (\$399 of \$2607) for basal only, compared with 22.2% for basal/bolus (\$812 of \$3666) and 15.1% for pre-mixed insulin (\$395 of \$2617).

Conclusion: This study shows that SMBG constitutes a large part of the treatment costs for insulin users. New generations of insulin used in regimens with simpler titration are warranted in order to enable relevant cost savings.

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Estimating the impact of different methods of utility assessment on the value of interventions in type 2 diabetes

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Background and aims: Methodological approaches to estimating utility in the presence of multiple co-morbidities include the 'minimum' approach, which employs the value of the condition with the lowest individual utility score and the 'multiplicative' approach, which employs the arithmetic product of utility scores as a proportion of full health. Health Technology Assessment (HTA) guidelines do not clearly recommend which method is preferred. This is of particular concern in the measurement of utility in diabetes as patients often experience multiple simultaneous complications. This investigation compares the estimated benefit of interventions using the multiplicative and minimum approaches to calculate patient utility.

Materials and methods: The IMS CORE Diabetes Model (CDM) is a well documented and validated computer simulation model for types 1 and 2 diabetes. The CDM was used to replicate two well known studies in type 2 diabetes, one investigating intensive glucose lowering therapy in newly diagnosed patients, average age 52 years (UKPDS cohort) and another in patients with more advanced disease, average age 62 years (ACCORD cohort). Expected quality adjusted life years (QALYs) in each arm and the benefit of therapy were calculated for each study using minimum and multiplicative utility calculation. To explore the possible impact on HTA we hypothesised an intervention that increased treatment cost by an arbitrary €5,000 over the patient's lifetime, and tested how much the incremental cost per QALY gained (CPQ) would be affected by the choice of utility calculation method. The model was run over a lifelong time horizon without discounting.

Results: Table: Expected outcomes in QALYs per patient treated

	UKPDS cohort			ACCORD cohort		
	Conven-tional therapy	Intensive therapy	Benefit	Conven-tional therapy	Intensive therapy	Benefit
QALYs per patient (minimum approach)	14.13	14.55	+0.42	8.09	8.34	0.25
QALYs per patient (multiplicative approach)	13.62	14.11	+0.49	7.35	7.68	0.33
Difference (multiplicative vs minimum, %)	-3.6%	-3.0%	+16.7%	-9.1%	-7.8%	+32.0%

The estimated CPQ for the hypothetical intervention in the UKPDS cohort was €11,900 using the minimum approach and €10,200 using the multiplicative approach and in the ACCORD cohort was €20,000 using the minimum approach and €15,150 using the multiplicative approach.

Conclusion: Compared with the minimum approach, the multiplicative approach generated lower estimates of total lifetime QALYs but larger estimated benefits from therapy. These effects are most pronounced in patients with more advanced disease at baseline. These differences are large enough to alter cost-effectiveness ratios appreciably and hence potentially impact the outcomes of HTA.

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Effects of basal bolus vs basal plus insulin regimens on weight in patients with type 2 diabetes

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Background and aims: Patients with type 2 diabetes (T2D) may require prandial insulin injections in addition to basal insulin, but optimal regimens for individual patients are difficult to determine.

Materials and methods: This sub-analysis of a randomized controlled study compared the 1-year efficacy and safety of insulin glargine (GLAR) plus 1, 2, or 3 daily injections of insulin glulisine (GLU) in patients with or without weight gain. Patients were grouped according to those who received 1 (n=114), 2 (n=128), or 3 (n=209) GLU injections in addition to GLAR and oral medications; results within each injection category were stratified by patients who gained weight vs those with no weight gain (or weight loss). Baseline demographics and clinical characteristics were similar among the 3 groups, except for a higher percentage of males and lower mean HbA_{1c} in the 1 GLU group vs groups 2 or 3.

Results: Baseline demographics were similar among groups, except for more males (49% vs 38% and 36%) and lower mean A1C (8.22% vs 8.47% and 8.53%) in group 1-GLU vs groups 2 or 3. Overall, at week 52, 3-GLU patients were more likely to reach A1C goal ($\leq 7.0\%$) vs 1-GLU patients, (26% vs 17%, $P=0.02$) but were not more likely to gain weight (1.9 kg vs. 1.3 kg, $P=NS$). At week 52, 38/114 (33.3%) and 66/209 (31.6%) patients in 1-GLU and 3-GLU, respectively, maintained or lost weight (mean[SD] 2.0[2.2] kg and 1.9[1.9] kg, respectively); 26.3% and 25.8% of these patients achieved A1C $\leq 7.0\%$ (Table). Patients receiving 1-GLU who maintained or lost weight had 3X-greater odds of achieving A1C $< 7.0\%$ vs patients who gained weight (26.3% vs 10.5%, $P=0.04$). Incidence and event rates of hypoglycemia with SMBG < 50 mg/dL were similar among the 3 groups (1-GLU, 2-GLU and 3-GLU incidence [%]: 21.9, 22.7, 15.3 and event rate [events/pt year]: 0.58, 1.04, 0.60, $P=NS$).

Conclusion: The results of this analysis suggest that patients with a basal plus insulin regimen can achieve glycemic goals while also maintaining or losing weight. Increasing the number of daily GLU injections from 1 to 3 does not significantly increase weight gain or risk of hypoglycemia.

	Glulisine x1		Glulisine x2		Glulisine x3	
	Δ wgt ≤ 0 kg n = 38	Δ wgt > 0 n = 76	Δ wgt ≤ 0 n = 37	Δ wgt > 0 n = 91	Δ wgt ≤ 0 n = 66	Δ wgt > 0 n = 143
Baseline HbA _{1c} , %	8.1 (0.9)	8.3 (1.1)	8.1 (0.8)	8.7 (1.3)	8.5 (1.1)	8.5 (1.1)
% Mean (SD)				$P < 0.01^*$		
52 week HbA _{1c} , %	7.8 (1.1)	8.3 (1.3)	7.5 (1.2)	8.0 (1.1)	7.9 (1.5)	7.8 (1.2)
% Mean (SD)				$P = 0.04^*$		$P = 0.03^*$
Change from baseline to 52 week HbA _{1c} , %	-0.25	0.01	-0.59	-0.69	-0.59	-0.74
% of patients achieving HbA _{1c} $< 7\%$ at week 52	26.3	10.5	40.5	13.2	21.2	22.4
% of patients achieving HbA _{1c} $\leq 7\%$ at week 52	26.3	11.8	40.5	15.4	25.8	25.9
				$P < 0.04^{**}$		
Baseline weight, kg	85.9 (18.2)	80.8 (17.1)	82.6 (16.9)	81.9 (16.0)	86.7 (15.1)	81.0 (15.0)
Mean (SD)						$P = 0.01^*$
52 week weight, kg	83.9 (17.7)	83.7 (17.7)	81.0 (16.5)	85.0 (16.7)	84.8 (14.8)	84.6 (15.4)
Mean (SD)						
Weight change from baseline to 52 week, kg, Mean (SD)	-2.0 (2.2)	2.9 (2.0)	-1.7 (2.1)	3.1 (2.2)	-1.9 (1.9)	3.6 (2.4)
		$P < 0.01^*$		$P < 0.01^*$		$P < 0.01^*$
Total Hypoglycemia incidence, %	50	42.1	54.1	59.3	43.9	45.5
				$P < 0.04^{**}$		
Hypoglycemia SMBG < 50 mg/dL, %	13.2	26.3	13.5	26.4	9.1	18.2

*P-value vs Δ wgt ≤ 0 .

[†]Logistic regression including weight gain category and baseline A1C as covariates.

^{**}P-value is for 2 vs 3 GLU injections, weight change > 0 .

Supported by: sanofi-aventis, US

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Short term impact of insulin therapy on cardiac function and lipid metabolism in patients with type 2 diabetes mellitus: a magnetic resonance imaging and spectroscopy study

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Background and aims: Patients with diabetes are at risk for specific myocardial disease (diabetic cardiomyopathy), which is causally related to increased myocardial lipid (MYCL) accumulation. In type 2 diabetes mellitus (DM2) intensive glucose lowering therapy has been associated with increased mortality of unknown cause. Thus, the aim of the present study was to assess whether induction of insulin therapy in patients with DM2 and insufficient metabolic control under oral anti-diabetic therapy affects cardiac lipid content and function.

Materials and methods: Magnetic resonance imaging and spectroscopy (MRI/MRS) measurements were performed twice in 9 patients with DM2 (4 female; age: 59 ± 10 years; BMI: 29.9 ± 5.1 kg/m²; HbA_{1c}: 11.2 ± 1.4 %) before and after 10 days of standardized insulin treatment. MYCL content and function were measured by ECG-triggered and breath movement navigated localized ¹H single voxel MRS and dynamic MRI, respectively. Intra-hepatic lipids were determined by ¹H MRS.

Results: Insulin therapy was associated with a 1,6-fold increase of MYCL (0.43 ± 0.46 vs. 0.69 ± 0.37 % of water signal; $p = 0.038$), while intra-hepatic lipid content did not change. In the short-term cardiac lipid accumulation was not associated with changes in left-ventricular systolic (ejection fraction [$p = 0.767$]; end-systolic volume [$p = 0.859$]) or diastolic (end-diastolic volume [$p = 0.953$]) function.

Conclusion: The induction of insulin therapy in patients with DM2 and bad metabolic control is associated with significant myocardial lipid accumulation but not with acute changes in left ventricular function. However, long term adverse effects of myocardial steatosis cannot be excluded.

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Mechanism of action of inhaled insulin Exubera[®] to normalise the fasting plasma glucose concentration in type 2 diabetic patients

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Aim: To examine the mechanism(s) via which Exubera, a “short acting” inhaled insulin administered three times daily, reduces the fasting plasma glucose (FPG) concentration which is primarily determined by the rate of endogenous (primarily hepatic) glucose production throughout the sleeping hours.

Methods: Ten T2DM subjects (age= 55 ± 3 yrs; BMI= 33 ± 1.4 , FPG= 211 ± 15 mg/dl; HbA_{1c}= 10.0 ± 1.2 %) completed 16 weeks of treatment with Exubera. At baseline and after 16 weeks subjects received: (i) an Oral Glucose tolerance test (OGTT) with plasma glucose, C-peptide and free fatty acid concentrations measured every 15 minutes, (ii) 24 hour plasma glucose profile using continuous glucose monitoring device (Medtronic, USA) (iii) hepatic glucose production ($3\text{-}^3\text{H-glucose}$).

Results: Following 16 weeks of Exubera therapy, body weight increased from 99 ± 5 to 101 ± 4 kg ($p < 0.05$). Fasting (211 ± 15 to 140 ± 11 mg/dl, $p < 0.005$) and 2-hour OGTT glucose (309 ± 9 to 264 ± 11 mg/dl, $p < 0.05$), the glucose AUC during OGTT, all decreased significantly ($34,847 \pm 1039$ to $28,586 \pm 1628$, $p < 0.05$). HbA_{1c} decreased from 10.0 ± 0.8 to 7.3 ± 0.7 , $p < 0.05$). The 24-hour plasma glucose profile was markedly decreased ($p < 0.001$) following Exubera treatment with a greater decline in the postprandial compared to fasting glucose. Fasting (0.662 ± 0.09 vs. 0.594 ± 0.08 mM) and post OGTT plasma free fatty acid concentration (0.319 ± 0.08 to 0.242 ± 0.08 mM) decreased significantly following treatment with Exubera. Plasma triglyceride (193 ± 47 to 103 ± 20) decreased, while plasma HDL concentration increased (33 ± 3.1 to 52 ± 5.3 , $p < 0.05$) following Exubera. LDL and total cholesterol did not change significantly. The basal rate of hepatic glucose production ($3\text{-}^3\text{H-glucose}$) decreased significantly (9.2 ± 0.9 vs. 6.9 ± 0.9 $\mu\text{mol/kg/min}$, $p = 0.03$) after 16 weeks of Exubera therapy. No significant difference was observed in measures of insulin secretion following 16 weeks of Exubera. There were no serious episodes of hypoglycemia and there was no change in FEV1 following treatment with Exubera (100 ± 4.7 vs. 96 ± 7 % of predicted).

Conclusion: Sixteen weeks of Exubera treatment caused a marked improvement in glycemic control, as evidenced by decreases in fasting glucose, 2-h glucose (OGTT), HbA_{1c}, and mean 24 hr glucose profile. The improvement in glycemic control is likely secondary to Exubera's effect in decreasing hepatic glucose production.

Supported by: Pfizer

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The BeAM factor: an easy-to-determine, objective, clinical indicator for when to add prandial insulin vs continued basal insulin titration

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Background and aims: In treating type 2 diabetes (T2D), it is often unclear when titration of basal insulin is maximized and prandial insulin should be added. We propose the adoption of an easily obtained measure to assist with such treatment decisions. Ideally, basal insulin therapy should match hepatic glucose production and maintain a narrow blood glucose range, including overnight. Large differences between Bedtime and AM (“BeAM” factor) blood glucose during treatment suggests that basal insulin may have reached maximal titration and that prandial insulin should be introduced to correct for PPG excursions to help achieve target HbA_{1c}.

Materials and methods: BeAM was examined using data pooled from 6 randomized controlled trials of insulin glargine vs a comparator added to oral antidiabetic drug (OAD) therapy in adults with T2D. Insulin was titrated to achieve FG ≤ 100 mg/dL. 1699 patients were included; 42% female, 95% white, mean age 59 (9) years, HbA_{1c} 8.7%, and duration of diabetes 9 (6) years.

Results: BeAM increased over 24 weeks of treatment (overall, BeAM increased from 25 mg/dL at baseline to 41 mg/dL at week 24, change of 16 mg/dL). Mean change in BeAM was greater in patients receiving basal insulin (Glargine or NPH, $n=1261$) vs other treatments (OADs or other insulin, $n=438$, mean change of 27 mg/dL vs -15 mg/dL, respectively, LS mean difference 36.5 mg/dL, $P < 0.0001$). Regardless of treatment, patients nearing target FG ≤ 100 mg/dL had an even greater increase in BeAM; of patients achieving pre-breakfast FG > 80 and < 120 mg/dL after 24 weeks ($n=431$), BeAM increased from 30 mg/dL at baseline to 58 mg/dL at week 24 (mean change of 32 mg/dL). The proportion of patients with BeAM > 50 mg/dL increased from 27% at baseline to 47% at week 24. In all 1699 patients and in those on basal insulin, a larger BeAM at week 24 was associated with reduced likelihood of attaining HbA_{1c} $\leq 7.0\%$ ($r^2=0.160$ all; $r^2=0.187$ BI; $P < 0.0001$ for both, Table) and increased risk of nocturnal ($r^2=0.152$, $P < 0.0001$), but not overall, hypoglycemia. Patients on basal insulin with a BeAM > 55 mg/dL were less likely to approach HbA_{1c} $\leq 7.0\%$. (Table)

Conclusion: This analysis suggests that patients on basal insulin with a BeAM factor > 55 mg/dL may not benefit from continued basal insulin titration and addition of prandial insulin should be considered to correct glucose excursions and achieve glycemic goals.

Relationship of BeAM and HbA_{1c} at 24 weeks

	HbA _{1c}	BeAM Least squares mean (SE)	P value difference from < 6.5
All patients (n=1699)	< 6.5	34.5 (2.65)	---
	≥ 6.5 to < 7.0	41.2 (2.57)	0.0570
	≥ 7.0 to < 7.5	46.0 (2.86)	0.0021
	≥ 7.5 to < 8.0	51.3 (3.46)	< 0.0001
	≥ 8.0	58.9 (3.56)	< 0.0001
Basal insulin patients (n=1261)	< 6.5	41.8 (3.06)	---
	≥ 6.5 to < 7.0	49.2 (2.95)	0.0685
	≥ 7.0 to < 7.5	54.9 (3.28)	0.0024
	≥ 7.5 to < 8.0	64.1 (4.05)	< 0.0001
	≥ 8.0	69.7 (4.22)	< 0.0001

Supported by: sanofi-aventis, US

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Achievement of glycaemic control by patients initiating basal insulinM. Aagren¹, N. Wu², P. Rao², L. Boulanger²;¹Novo Nordisk, Princeton, USA, ²United BioSource Corporation, Lexington, USA.

Background and aims: While many patients with type 2 diabetes mellitus (T2DM) achieve glycaemic goals with lifestyle modifications and the use of oral antidiabetic drugs (OADs), most patients will eventually require the inclusion of insulin in their treatment regimen to reach adequate control. This study identified T2DM patients initiating basal insulin and assessed the proportion of patients treated to goal and the time to reach goal.

Materials and methods: Electronic medical records (EMR) dated January 2005 through August 2010 were assessed from the General Electric EMR Database. Patients with T2DM who initiated basal insulin between July 2005 and August 2009 were selected, with initiation defined as no prescription record of insulin in the prior 15 months. Patients were grouped into five cohorts (glycated hemoglobin [A1C] $\leq 6.5\%$, $6.5\% < A1C \leq 7\%$, $7\% < A1C \leq 8\%$, $8\% < A1C \leq 9\%$, and $A1C > 9\%$) based on A1C measured within 90 days prior or up to 14 days following insulin initiation. Demographic characteristics, comorbidities and OAD use were assessed based on data from the 15 months prior to insulin initiation. The proportion of patients achieving A1C $\leq 7\%$ ("goal") within one year after initiation were estimated; the average time to achieve goal was estimated using the Kaplan-Meier method. Changes of A1C levels pre- and post- insulin initiation were calculated; statistical significance of difference from 0 was detected by pair-wise t-tests. Differences in means between cohorts were detected by analysis of variance tests, and trends in the proportions across A1C cohorts were detected by Mantel-Haenszel tests.

Results: Of the 13,494 T2DM patients who initiated basal insulin, 52.0% were 60+ years old and 50.6% were female. At baseline, more than half of the patients had A1C $> 8\%$, and 60% were obese. Sulfonylureas were the most commonly used OADs (53.4%), followed by metformin (52.6%). Usage increased significantly ($p < 0.01$) with increasing baseline A1C. Most patients (88.3%) initiated basal analogs (insulin detemir or insulin glargine), 11.0% initiated NPH, and 0.7% received prescriptions for both. The proportion of patients initiating NPH decreased from 19.0% to 8.1% across the A1C cohorts ($p < 0.01$). During the 12 months post insulin initiation, the proportion reaching goal decreased from 88.4% for the A1C ≤ 6.5 cohort to 29.0% for the A1C > 9 cohort, while average time to achieve goal increased from 199 days (95% confidence interval [CI]: 194–204) to 313 days (95% CI: 310–315) (Log-rank test: $p < 0.01$). After basal insulin initiation, mean A1C decreased significantly, by 0.41% (standard deviation [SD]: 0.96, $p < 0.01$) to 2.8% (SD: 2.3, $p < 0.01$) in cohorts with baseline A1C $> 7\%$.

Conclusion: After initiating basal insulin, a higher proportion of patients with T2DM with a lower baseline A1C reached the target of A1C $\leq 7\%$, and took fewer days to do so. Among patients whose baseline A1C were below target, the natural drift in A1C may offset the treatment effect of basal insulin, and thus result in a follow-up A1C above the 7% goal in some cases.

	Cohorts by baseline A1C value					p value
	All patients (n=13,494)	A1C ≤ 6.5 (n=1,228)	6.5 < A1C ≤ 7 (n=1,049)	7 < A1C ≤ 8 (n=3,178)	8 < A1C ≤ 9 (n=2,737)	A1C > 9 (n=5,302)
Mean age (SD)	60.0 (12.6)	64.0 (13.0)	64.4 (12.7)	63.0 (11.9)	60.9 (12.0)	56.0 (12.0)
Female (%)	50.6	52.4	54.1	52.4	49.6	49.0
Initiated NPH (%)	11.0	19.0	18.4	11.5	9.6	8.1
Initiated Analog (%)	88.3	80.6	81.1	87.8	89.7	91.2
Prior use of Sulfonylureas (%)	53.3	31.1	42.2	56.6	60.8	54.7
Prior use of Metformin (%)	52.6	37.6	42.4	53.0	57.0	55.7
Achieved A1C $\leq 7\%$ within 12 months since initiation (%)	43.6	88.4	70.2	51.2	32.9	29.0
Average days from insulin initiation to goal (95% CI)	287 (285–289)	199 (194–204)	233 (226–239)	277 (273–281)	308 (305–312)	313 (310–315)
Average change of A1C value (SD)	-1.35* (2.01)	0.20* (0.80)	0.01 (0.95)	-0.41* (0.96)	-0.93* (1.10)	-2.80* (2.30)

*Significantly different across cohorts by Log-rank test.

*Significantly different from 0 by pair-wise t-test.

Difference in means between cohorts was detected by Analysis of Variance test and trends in proportions across cohorts were detected by Mantel-Haenszel tests.

Supported by: Novo Nordisk

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Outcomes associated with insulin therapy discontinuation after hospital discharge in patients with type 2 diabetes initiated on insulin during hospitalisationS. Zhou¹, E. Wu², A. Peng Yu², M. Lu³, H. Sharma², T. Graf³;¹sanofi-aventis US, Bridgewater, ²Analysis Group, Boston, ³Geisinger Health System, Danville, USA.

Background and aims: In the real world, regardless of HbA_{1c} levels during hospitalization, patients may discontinue insulin therapy (INS) after hospital discharge. However, there is a lack of understanding on the clinical outcomes

associated with insulin discontinuation post hospital discharge. This study investigated the clinical outcomes and risk of hospital re-admission associated with discontinuation of INS after hospital discharge among patients with type 2 diabetes (T2DM) who initiated INS during hospitalization.

Materials and methods: Adult T2DM patients newly initiated on INS during a hospitalization were identified in MedMining Electronic Health Record data (from 01/2004 to 04/2010). Patients with a last recorded HbA_{1c} $\geq 8\%$ within 3 months of (or during) the hospital stay were included. Outcomes were compared between patients with INS continuation after the hospitalization (defined as an outpatient INS order within 60 days post-discharge) vs. patients with INS discontinuation. During the 12-month post-discharge, changes in HbA_{1c} were compared using t-tests and regression models. HbA_{1c} goal achievement ($< 7\%$) and hypoglycemia were evaluated using chi-square tests and logistic regression models. Time to re-admission was compared using Cox proportional hazard models. All regression models controlled for patient characteristics prior to and during hospitalization and concurrent medications received within 60 days of discharge. A subgroup analysis was performed in patients with baseline HbA_{1c} $\geq 9\%$.

Results: 180 patients with INS continuation and 552 patients with INS discontinuation were included (baseline HbA_{1c} 11.1% vs. 9.5%, respectively). During the 12-months post-discharge, patients continuing INS had greater HbA_{1c} reduction (3.4% vs. 1.5%, $P < 0.01$), higher HbA_{1c} goal achievement (41% vs. 31%, $P = 0.02$), and comparable hypoglycemia (8% vs. 5%, $P = 0.25$) vs. those with INS discontinuation. In the HbA_{1c} $\geq 9\%$ subgroup similar patterns were observed for INS continuation ($N = 146$) vs. discontinuation ($N = 277$) (baseline HbA_{1c}: 11.7% vs. 10.6%; HbA_{1c} reduction: 4.1% vs. 2.3%, $P < 0.01$; HbA_{1c} goal achievement: 44% vs. 30%, $P = 0.02$; hypoglycemia: 6% vs. 5%, $P = 0.75$). Multivariate analysis were generally consistent with descriptive findings for both the HbA_{1c} $\geq 8\%$ and $\geq 9\%$ groups (HbA_{1c} reduction: adjusted difference = 1.67% and 1.53%, both $P < 0.01$; HbA_{1c} goal achievement: odds ratio [OR] = 1.66 and 2.15, $P = 0.06$ and $P = 0.03$; hypoglycemia: OR = 1.02 and 0.91, $P = 0.97$ and 0.90; respectively). Among the HbA_{1c} $\geq 9\%$ subgroup, INS continuation was associated with lower risks of all-cause and diabetes-related hospitalizations (hazard ratio: 0.58 and 0.46 respectively; both $P < 0.05$).

Conclusion: T2DM patients who continued INS after hospital discharge had a greater HbA_{1c} reduction, a greater HbA_{1c} goal achievement, and a lower risk of hospital re-admission compared with patients with discontinued INS. Supported by: sanofi-aventis US

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Outcomes following insulin therapy disruption after hospital discharge in patients with type 2 diabetesE. Wu¹, S. Zhou², A. Peng Yu¹, M. Lu¹, H. Sharma¹, T. Graf²;¹Analysis Group, Boston, ²sanofi-aventis US, Bridgewater, ³Geisinger Health System, Danville, USA.

Background and aims: In the real world setting, hospitalization may disrupt insulin therapy (INS) and lead to suboptimal care. However, there is a lack of understanding on the clinical outcomes associated with insulin disruption post hospital discharge. This study investigated clinical outcomes and urgent care use following disruption of INS after hospital discharge among patients with type 2 diabetes mellitus (T2DM) who had used INS before and during the hospital stay.

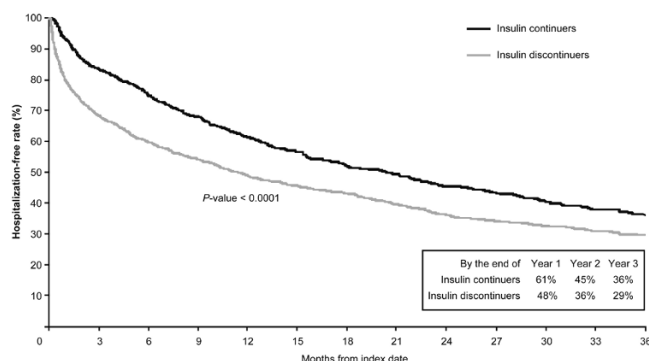
Materials and methods: 2,160 adult T2DM patients were identified with INS use within 30 days before and during a hospital stay from electronic medical records in a coordinated health care system (01/2004 to 04/2010). Outcomes were compared between 851 patients with vs. 1,309 patients without INS continuation over the first 60 days post-discharge.

Results: Compared with patients with INS disruption, patients who continued INS were younger (63 vs. 65 years, $P < 0.01$), had more frequent baseline ophthalmic complications (36% vs. 29%, $P < 0.01$), and higher blood glucose on admission (11.7 mmol/L vs. 10.4 mmol/L, $P < 0.01$). Kaplan-Meier analysis showed that patients who continued INS had significantly lower risks of all-cause re-admission compared with patients with INS disruption, which was confirmed using Cox models (see figure). INS continuation was associated with significantly lower risk of diabetes-related re-admissions (hazard ratio [HR]: 0.88; 95%CI: 0.77, 0.99) and ER visits (HR: 0.88; 95%CI: 0.78, 0.99). Patients who continued INS had greater HbA_{1c} reduction within 1 year after discharge vs. those with INS disruption (0.51% vs. 0.17%, $P < 0.01$). A similar trend was observed among patients with HbA_{1c} $\geq 7\%$ before discharge (0.75% vs. 0.42%, $P < 0.01$) which consists of 71% of the population with HbA_{1c} values. Multivariate regression analysis showed that patients with INS continuation

had a significantly higher HbA_{1c} reduction, both in the overall population (diff=0.31%, $P<0.01$) and in those patients with HbA_{1c} $\geq 7\%$ (diff=0.29%, $P<0.05$).

Conclusion: T2DM patients who continued INS after hospital discharge had greater HbA_{1c} reduction and lower risk of urgent care visits vs. patients who disrupted INS.

Kaplan-Meier curves for time to the first all-cause hospital re-admission among T2DM patients continuing vs. those disrupting insulin



Interpretation: Insulin continuers have a significantly lower risk of all-cause hospital re-admission compared with insulin discontinuers after discharge (adjusted hazard ratio: 0.82 [95%CI: 0.73, 0.93]).

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Clinical characteristics and outcomes in patients with type 2 diabetes adding insulin glargine to exenatide or exenatide to insulin glargine in a US managed care setting

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Background and aims: Few studies have addressed the real-world efficacy or treatment persistence of insulin glargine (GLA) and exenatide (EX) in combination. This study evaluated the clinical characteristics and outcomes in patients with type 2 diabetes mellitus (T2DM) when these medications were used in combination.

Materials and methods: Using a national US insurance claims database, a retrospective study was conducted in T2DM patients aged ≥ 18 years old, who added EX to GLA (EX+GLA) or GLA to EX (GLA+EX) from 2006 to 2009 and had continuous health plan coverage 6-month pre- (baseline) and 1-year post-index (follow-up).

Results: 422 patients were included, with the majority (67%, $n=281$) in the EX+GLA cohort (mean age 53.9 yrs; 52.3% male) and 33% ($n=141$) in the GLA+EX cohort (mean age 54.2 years; 58.2% male). The EX+GLA cohort had lower HbA_{1c} than the GLA+EX cohort at baseline (see table). A significant reduction in HbA_{1c} was observed in both cohorts at follow-up. Average daily dose of glargine was 36.1 units in the GLA+EX and 43.4 units in the EX+GLA group. The mean number of hypoglycemic events increased slightly from baseline but remained low in the EX+GLA and GLA+EX cohorts (0.25 to 0.75 and 0.17 to 0.57 events per patient per year, respectively). Improved lipid profiles were observed at follow-up in both cohorts. Only small proportions of patients stayed on both drugs at the end of 1-year follow-up (EX+GLA: 11% and GLA+EX: 9.9%) but more patients stayed on GLA than EX in both cohorts (EX+GLA: 43.6% vs. 21.0%; GLA+EX cohorts: 44.0% and 23.6%, respectively).

Conclusion: This real-world study demonstrated that combination use of GLA and EX in T2DM patients with poor glycemic control was associated with significant reductions in HbA_{1c} with no significant increase in the frequency of hypoglycemia.

	EX+GLA (n=281)			GLA+EX (n=141)		
	Baseline	Mean change at end of follow-up	P-value	Baseline	Mean change at end of follow-up	P-value
HbA _{1c} , mean \pm SD	8.4 \pm 1.5	-0.4 \pm 1.5	<0.0001	8.9 \pm 1.6	-0.9 \pm 1.6	<0.0001
HDL-C, mg/dL, mean \pm SD ¹	41.7 \pm 12.0	-2.2 \pm 10.7	0.0665	41.7 \pm 15.9	1.1 \pm 12.3	0.53
LDL-C, mg/dL, mean \pm SD ¹	92.2 \pm 35.7	-15.2 \pm 36.9	0.0003	84.5 \pm 36.1	-6.8 \pm 29.4	0.12
TG, mg/dL, mean \pm SD ¹	211.4 \pm 168.0	-18.5 \pm 91.5	0.0699	206.7 \pm 171.9	-59.3 \pm 169.8	0.02

GLA, insulin glargine; EX, exenatide

¹For lipids subset, $n=92$ -95 for EX+GLA and $n=55$ for GLA+EX

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Baseline characteristics from 11 prospective randomised trials that predict an effective approach to starting and adjusting insulin glargine

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Background and aims: Insulin glargine (GLAR) can be added to patients with type 2 diabetes (T2DM) inadequately controlled by oral agents, yet the most effective and safe starting dose or the maximum dose to reach endpoint glucose targets in a given patient is unclear. This analysis examined whether baseline characteristics can predict appropriate GLAR starting and final dosages in T2DM patients.

Materials and methods: Data were pooled from 11 prospective, 24-week, randomized, controlled trials ($N=2311$) using basal GLAR as the only insulin. Strict, predefined insulin titration algorithms were followed to achieve FPG ≤ 100 mg/dL (5.6 mmol/L).

Results: Mean age was 58.6 years, 55.8% were male, and 81.6% were white. The Table shows Week 24 weight-adjusted insulin doses, % of patients achieving HbA_{1c} $\leq 7\%$, and yearly rates of severe hypoglycemia. GLAR requirements increased in obese ($BMI > 30$) vs non-obese patients and in patients with greater baseline HbA_{1c}. GLAR requirements decreased in elderly (≥ 65 yrs) vs non-elderly (< 65 yrs) patients. Week 24 target HbA_{1c} ($\leq 7\%$) was reached by $\sim 65\%$ with baseline HbA_{1c} $< 9\%$; $\sim 40\%$ with HbA_{1c} $\geq 9\%$. Yearly event rates of severe hypoglycemia were generally low in all groups; rates were slightly higher with higher baseline HbA_{1c}. In the elderly, a 0.1 U/kg starting dose can be safely titrated to 0.3-0.4 U/kg to reach target endpoints. In the nonelderly, if HbA_{1c} is $< 9\%$ start with 0.1 U/kg and adjust to 0.4 U/kg; if HbA_{1c} is $\geq 9\%$ start with 0.2 U/kg and adjust to 0.5-0.6 U/kg.

Conclusion: While GLAR dosages should be determined by the physician based on individual patient characteristics, these baseline predictor data from a large patient pool provide clinically relevant guidelines for starting and adjusting GLAR dosages that allow patients to reach glycemic targets safely (minimizing severe hypoglycemia).

Table.				
	<65 years		≥ 65 years	
Baseline HbA _{1c}	Non-obese	Obese	Non-obese	Obese
Week 24 weight-adjusted insulin dose (U/kg) (Mean [SD])				
<9%	0.38 (0.22)	0.45 (0.25)	0.33 (0.19)	0.34 (0.17)
$\geq 9\%$	0.49 (0.25)	0.58 (0.29)	0.41 (0.21)	0.39 (0.20)
Week 24 HbA _{1c} $\leq 7\%$ (n/N, %)				
<9%	260/388 (67.0)	357/516 (69.2)	149/235 (63.4)	106/158 (67.1)
$\geq 9\%$	109/286 (38.1)	146/365 (40.0)	53/129 (41.1)	36/93 (38.7)
Severe hypoglycemia (events/year) (Mean [SD])				
<9%	0.02 (0.18)	0.08 (0.62)	0.07 (0.80)	0.03 (0.25)
$\geq 9\%$	0.15 (1.87)	0.01 (0.16)	0.00 (0.00)	0.02 (0.23)

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Proportion of patients with type 2 diabetes achieving HbA_{1c} targets without weight gain or hypoglycaemia in PREDICTIVE™: the value of earlier initiation or intensification of insulin therapy

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Background and aims: Hypoglycaemia and weight gain frequently act as barriers to modification of regimen in type 2 diabetes (T2D), and may more than offset the benefits of HbA_{1c} reduction. Yet early action before blood glucose levels rise substantially above target levels may reduce the risk of these unwanted side effects.

Materials and methods: PREDICTIVE was a multi-national observational study evaluation of insulin detemir (IDet) use in routine clinical practice. Data from 11 European countries sub-divided by starting and subsequent regimen were used in an exploratory logistic regression analysis to identify predictors for a composite endpoint of HbA_{1c} <7% (53 mmol/mol), without weight gain or hypoglycaemia after 3 months of treatment with IDet. Logistic regression using a multivariate analysis was made using the baseline characteristics: diabetes duration; age; BMI; frequency of hypoglycaemia 4 weeks prior to study start; HbA_{1c}; and fasting blood glucose (FBG).

Results: Observations from patients with efficacy data (n=6979, missing data=255) were included in the analysis; of these patients, 1408 (21%) achieved the composite endpoint. The Table illustrates that indicators of glycaemic control (baseline HbA_{1c} and FPG) were significant predictors for patients <7% (53 mmol/mol) without side-effects when using IDet. Similarly, although to a lesser extent, BMI and experience of hypoglycaemia at baseline were also predictors, as was the duration of diabetes. Directionally, patients with lower HbA_{1c} on entering the study who then received regimen modification (either initiation or switch of insulin to IDet) benefitted more than those for whom HbA_{1c} was higher. Similarly, diabetes duration correlated with this trend, with early intervention again bringing greater benefits - lower BMI and less frequent hypoglycaemia were similarly predictors of greater benefits.

Conclusion: These data support the earlier initiation of IDet or modification of a regimen which is failing to control blood glucose levels; more timely intervention by physicians (defined here as intervention before HbA_{1c} levels rise beyond recommended target levels) may enable their patients to achieve or maintain HbA_{1c} <7% (53 mmol/mol) without weight gain or hypoglycaemia.

Table. Predictors of tight glycaemic control without hypoglycaemia or weight gain

Factor	Mean (SD)	Logistic regression p-value
Gender (female:male)	4149:3732 (53% female)	0.80
Age (years)	61.0 (10.7)	0.65
Duration of diabetes (years)	10.8 (7.2)	<0.0001
BMI (kg/m ²)	30.9 (5.7)	0.0005
HbA _{1c} (%; mmol/mol)	8.46 (1.58)% 69 (6)	<0.0001
FBG (mmol/l)	10.33 (3.13)	<0.0001
Total number of hypoglycaemic events	0.66	0.0021

Key: hypoglycaemic events were recorded as 'n' based on patient recall from the four weeks prior to baseline. All p-values refer to multivariate model logistic analysis data

Clinical Trial Registration Number: NN304-1677

Supported by: Novo Nordisk

PS 091 Insulin therapy in type 2 diabetes II

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Biphasic insulin aspart 30 and insulin glargine administered with oral anti-diabetic drugs in type 2 diabetes mellitus: a systematic review and meta-analysis

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Background and aims: There is an on-going debate whether the addition of biphasic insulin analog to oral anti-diabetic drugs (OADs) provides any clinical advantages over basal insulin added to OADs in patients with type 2 diabetes mellitus (T2DM). A systematic review-based study was performed to compare the clinical outcomes of treatment with biphasic insulin aspart 30 (BIAsp 30) and insulin glargine (IGlar) in T2DM patients inadequately controlled with OADs.

Materials and methods: The analysis was based on randomized controlled trials (RCTs) identified by a systematic literature search in medical databases (MEDLINE, EMBASE, The Cochrane Library and others) up to February 2011. Studies met the inclusion criteria if they compared BIAsp 30 vs. IGlar added to at least one OAD in T2DM patients. It should be noted that in some studies, patients in the BIAsp 30 group received different OADs than those from the IGlar group. Results were presented as the weighted mean difference (WMD) or odds ratio (OR) with a 95% confidence interval.

Results: We identified 6 trials including together 1422 patients followed from 24 to 28 weeks. A meta-analysis of these 6 RCTs demonstrated that BIAsp 30 reduced HbA_{1c} level more effectively than IGlar (WMD = -0.23 % [-0.40, -0.05]). In addition, statistically significant differences were observed in favour of BIAsp 30 for mean prandial glucose increment (3 RCTs; WMD = -14.70 mg/dl [-20.09, -9.31]); but not for fasting plasma glucose (4 RCTs; WMD = 6.83 mg/dl [-12.76, 26.43]). Pooled data of 4 RCTs showed that the percentage of patients with at least one hypoglycaemic episode was higher in the BIAsp 30 group than in the IGlar group (61% vs. 50%; OR = 1.59 [1.22, 2.07]). However, no significant differences were revealed in respect to severe hypoglycaemic episodes (6 RCTs; 1% vs. 1%; OR = 0.84 [0.33, 2.15]) and premature discontinuation (5 RCTs; 12% vs 11%; OR = 1.23 [0.68, 2.25]). There was no difference in weight gain between the groups (5 RCTs; WMD = 0.41 kg [-1.16, 1.98]).

Conclusion: When compared to IGlar, BIAsp 30 added to OAD treatment resulted in better glycaemic control in T2DM patients and did not increase risk of severe hypoglycaemia.

Supported by: Novo Nordisk Poland

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Insulin detemir versus insulin glargine for type 2 diabetes mellitus

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Background and aims: Chronically elevated blood glucose levels are associated with significant morbidity and mortality. Many diabetes patients will eventually require insulin treatment to maintain good glycaemic control. There are still uncertainties about the optimal insulin treatment regimens for type 2 diabetes, but the long-acting insulin analogues seem beneficial. Several reviews have compared either insulin detemir or insulin glargine to NPH insulin, but research directly comparing both insulin analogues is limited. The aim of the study is to assess the effects of insulin detemir and insulin glargine compared with each other in the treatment of type 2 diabetes mellitus.

Materials and methods: We searched MEDLINE, EMBASE, The Cochrane Library, online registries of ongoing trials and abstract books. All randomised controlled trials (RCTs) comparing insulin detemir with insulin glargine with a duration of 12 weeks or longer were included.

Results: This review examined 4 trials lasting 24 to 52 weeks involving 2250 people randomized to either insulin detemir or glargine. Insulin glargine was dosed once-daily in the evening. Insulin detemir was initiated once-daily in the evening with the option of an additional dose in the morning in three studies and initiated twice-daily in one study. 13.6 to 57.2% of patients were

injecting insulin detemir twice-daily at end of trial. Glycaemic control, measured by glycosylated haemoglobin A1c (HbA_{1c}), FPG (fasting plasma glucose) and HbA_{1c} ≤ 7% with/without hypoglycaemia, did not differ significantly between treatment groups, except for one outcome measure; insulin glargine was associated with lower FPG at study endpoint. The results showed no differences in overall, nocturnal and severe hypoglycaemia between treatment groups. Insulin detemir was associated with less weight gain. Treatment with insulin glargine resulted in a lower daily basal insulin dose and a lower number of injection site reactions. There was no difference in the variability of FPG or glucose values in 24-hour profiles between treatment groups. It was not possible to draw conclusions on quality of life, costs or mortality.

Conclusion: Our analyses suggest that there is no clinically relevant difference in efficacy or safety between insulin detemir and insulin glargine for targeting hyperglycaemia. However, to achieve the same glycaemic control insulin detemir was often injected twice-daily in a higher dose but with less weight gain, while insulin glargine was injected once-daily, with somewhat fewer injection site reactions.

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Basal-bolus therapy with insulin degludec improves long-term glycaemic control with fewer nocturnal hypoglycaemic events compared with insulin glargine in people with type 2 diabetes

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Background and aims: Upon subcutaneous injection, insulin degludec (IDeg), a new-generation basal insulin, forms soluble multi-hexamers which dissociate slowly to produce an ultra-long and flat action profile. This 1-year, open-label, treat-to-target trial compared the efficacy and safety of IDeg with insulin glargine (IGlar), both administered once daily with mealtime insulin aspart (IAsp) ± metformin ± pioglitazone as part of a basal-bolus treatment regimen.

Materials and methods: 992 subjects (mean: age 58.9 years; diabetes duration 13.5 years; HbA_{1c} 8.3%; FPG 9.2 mmol/l) with type 2 diabetes and HbA_{1c} 7–10% after ≥ 3 months of any insulin regimen ± OAD(s), randomised (3:1) to IDeg or IGlar were analysed. Basal and bolus insulin were titrated weekly throughout the study using structured algorithms. The target glucose for basal insulin was an FPG < 5 mmol/l.

Results: A similar proportion of subjects completed the trial with IDeg (83%) and IGlar (85%). At the end of the study, IDeg and IGlar improved overall HbA_{1c} levels by 1.2 %-points and 1.3 %-points, respectively (estimated treatment difference (ETD) IDeg–IGlar: 0.08 %-points [95% CI: –0.05, 0.21]), and half of the subjects in both groups achieved HbA_{1c} < 7% (p=NS). FPG was reduced by 2.4 mmol/l with IDeg and by 2.1 mmol/l with IGlar (ETD: –0.29 mmol/l [95% CI: –0.65; 0.06], p=NS). The rates of overall confirmed hypoglycaemia (defined as episodes with PG < 3.1 mmol/l or considered severe according to ADA definition) were significantly lower with IDeg than IGlar (11.1 vs. 13.6 episodes/patient-year; estimated rate ratio (ERR) IDeg/IGlar: 0.82 [95% CI: 0.69; 0.99], p=0.0359). Similarly, the rate of nocturnal confirmed hypoglycaemia, (confirmed hypoglycaemia occurring between 00:01–05:59) was 25% lower in the IDeg group compared with the IGlar group (1.4 vs. 1.8 episodes/patient-year; ERR: 0.75 [95% CI: 0.58; 0.99] p=0.0399). IDeg was well tolerated; the rates of adverse events were similar between groups and there were no treatment-specific patterns. By the end of the study, total mean daily insulin doses were 1.46 U/kg and 1.42 U/kg in the IDeg and IGlar groups, respectively. In both groups, the relative contribution of the basal and bolus components was approximately 50:50.

Conclusion: Insulin degludec, given as basal-bolus treatment with insulin aspart in people with type 2 diabetes, improves long-term glycaemic control with a significantly lower risk of overall and nocturnal hypoglycaemia compared with insulin glargine.

Clinical Trial Registration Number: NCT00972283

Supported by: Novo Nordisk

1036

Comparison of 3 intensified insulin regimens added to oral therapy for type 2 diabetes: twice-daily aspart premixed vs glargine plus 1 prandial glulisine or stepwise addition of glulisine to glargine

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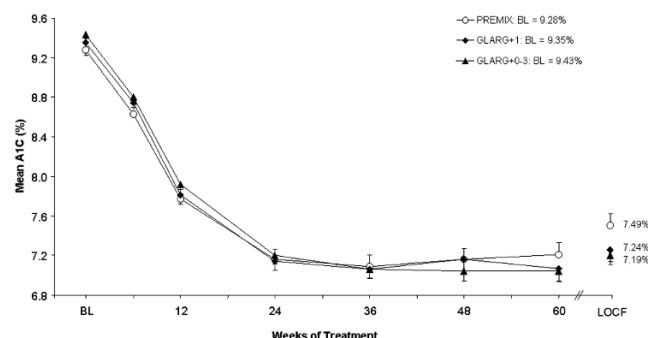
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Background and aims: How to advance from unsuccessful oral antidiabetic therapy to intensified insulin therapy in type 2 diabetes is debated.

Materials and methods: A 60-week randomized, open-label study compared efficacy, hypoglycemia, and body-weight after adding BID premixed 70/30 protamine-aspart/aspart (PREMIX, n=192), glargine + 1 prandial glulisine dose (GLARG+1, n=189), or glargine plus stepwise glulisine (GLARG+0-3, n=191).

Results: Mean baseline HbA_{1c} was 9.4% after a 4-week run-in on 2-3 oral agents; age 54 years, diabetes duration 9 years, BMI 33.2 kg/m². Insulin was titrated seeking fasting and preprandial glucose < 100 mg/dL (5.6 mmol/L) with HbA_{1c} < 6.5%. Each regimen reduced HbA_{1c} (Figure) but more randomized patients had week 60 HbA_{1c} < 7% with GLARG+1 (49%, P<0.025) or GLARG+0-3 (45%, P<0.05) than with PREMIX (39%), and more did so without hypoglycemia with GLARG+1 (24%, P<0.05) or GLARG+0-3 (24%, P<0.01) than with PREMIX (14%). Mean ΔHbA_{1c} at endpoint (last observation carried forward [LOCF]) was –1.8±0.1% with PREMIX vs –2.1±0.1% (P=0.06) and –2.2±0.1% (P<0.01) with GLARG+1 and GLARG+0-3. Adjusted event-rates per patient-yr (ER) and incidences (%) for categories of hypoglycemia are reported below. Δ in body weight at week 60 was similar among regimens.

Conclusion: Significantly larger proportions of patients achieved target HbA_{1c} < 7% on both glargine-based regimens with less hypoglycemia than with premixed insulin.



	PREMIX		GLARG+1		GLARG+0-3	
	ER	%	ER	%	ER	%
BG < 70 mg/dL (3.9 mmol/L) + symptoms	12.2±1.7	724	7.1±1.0*	634*	7.2±1.0*	604*
BG < 50 mg/dL (2.8 mmol/L) + symptoms	1.9±0.3	464	0.8±0.2*	334*	0.9±0.2*	324*
BG < 35 mg/dL (2.0 mmol/L)	0.2±0.1	144	0.1±0.0*	94	0.2±0.1	114
Requiring assistance	0.2±0.1	94	0.1±0.0	74	0.2±0.1	104
Coma or seizure		24	0			0.4±0.4

ERR: *p<0.05, **p<0.01, ***p<0.001 vs PREMIX

Clinical Trial Registration Number: NCT00384085

Supported by: sanofi-aventis, US

1037

Randomised open-label trial of insulin lispro protamin suspension versus insulin glargine once daily in basal-bolus therapies with insulin lispro in type 2 diabetes patients

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Background and aims: This study compares the efficacy and safety of insulin lispro protamine suspension (ILPS) versus insulin glargine once daily in a basal-bolus regimen in patients with type 2 diabetes mellitus (T2DM) who no longer achieved glycemic control with combination treatment consisting of insulin and oral antidiabetic medication (OAM).

Materials and methods: In this 24-week, open-label, parallel group study, adult insulin-pretreated T2DM patients were randomized to receive a basal-bolus regimen of once daily ILPS or insulin glargine, plus insulin lispro before main meals 2 to 3 times a day. Main inclusion criteria were OAM treatment, HbA_{1c} 7.5% to 11.0%, BMI 25 to 45 kg/m². All OAMs except metformin were discontinued at randomization. Insulin doses were titrated to predefined blood glucose (BG) targets over 12 weeks. Non-inferiority of ILPS versus glargine was assessed by comparing the upper limit of the 95% confidence interval (CI) for the change of HbA_{1c} from baseline to Week 24 (adjusted for country and baseline HbA_{1c}) with a non-inferiority margin of 0.4%. Non-inferiority was assessed on the per-protocol population; other analyses were performed on the full analysis set. Secondary efficacy and safety variables included HbA_{1c} categories, BG profiles, insulin doses, hypoglycemic episodes, adverse events, and vital signs.

Results: 383 patients were randomized (age 33–86 years; 43% male) to receive ILPS (n=192) or insulin glargine (n=191). Non-inferiority of ILPS versus glargine in the change of HbA_{1c} from baseline to 24 weeks was shown (Table). HbA_{1c} targets of <7.0% were achieved by 21.7% (ILPS) versus 29.4% (glargine) of patients. No clinically relevant differences between treatments were observed in self-monitored BG profiles at 24 weeks. Mean basal/meal-time insulin doses at Week 24 were 29.6/36.2 IU/day (ILPS) versus 32.8/42.2 IU/day (glargine); the difference was not statistically significant for total dose at Week 24 (p=0.7). 56.1% (ILPS) versus 63.6% (glargine) of patients experienced any hypoglycemia (p=0.2), 25.7% (ILPS) versus 19.3% (glargine) experienced nocturnal hypoglycemia (p=0.2). No clinically relevant differences were noted in any other variables.

Conclusion: A basal-bolus regimen with ILPS once daily resulted in non-inferior glycemic control compared to a similar regimen with glargine, without statistically significant or clinically relevant differences in hypoglycemia. ILPS-based regimens can be considered an alternative to basal-bolus regimens with glargine for T2DM patients.

Change in HbA_{1c} from Baseline to Week 24 - Analysis of Covariance

	ILPS				Glargine				Difference ILPS minus Glargine	
	N	LS mean	95% CI		N	LS mean	95% CI		LS mean	95% CI
Population										
PP set	145	-1.18	-1.36; -1.00		157	-1.28	-1.44; -1.11		0.10	-0.11; 0.31
(primary)										
FAS	166	-1.16	-1.33; -0.99		170	-1.27	-1.43; -1.11		0.11	-0.09; 0.31

CI = confidence interval; FAS = full analysis set; ILPS = insulin lispro protamine suspension; LS = least square; N = number of evaluable patients; PP = per protocol.

Statistics from Analysis of Covariance model (complete case analysis): HbA_{1c} change from baseline = Treatment + country + baseline HbA_{1c} value.

Clinical Trial Registration Number: NCT00666718

Supported by: Eli Lilly and Company

1038

Efficacy and safety of a fixed dose combination of glimepiride and metformin versus glimepiride when added in to insulin glargine in patients with type 2 diabetes

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Background and aims: Patients (pts) with type 2 diabetes mellitus (T2D) with non-controlled blood glucose level are recommended to be treated and initiated with aggressive oral combination therapy and early insulin therapy. The aim of this study was to evaluate if a fixed dose combination of glimepiride 1mg and metformin 500mg twice daily (GMet) is noninferior to glimepiride 4mg (G) once daily in change in HbA_{1c} when added to insulin glargine in adults with suboptimally controlled T2D.

Materials and methods: This open-label, multicenter, randomised, 16-week trial enrolled pts with T2D (BMI ≥ 21 kg/m² and ≤ 11 kg/m², HbA_{1c} > 7.0% and < 11% who had been treated ≥ 3 months with glimepiride and metformin. Pts were randomly assigned to receive either GMet (n=49) or G (n=48). Statistical analysis was performed based on intent-to-treat population using last observation carried forward method. The effectiveness of GMet (vs G) was assessed

hierarchically by a non-inferiority comparison, with a margin of 0.4%, and then by a superiority comparison.

Results: A total of 97 patients were randomized. Baseline demographics were similar across treatment groups. At 26-week endpoint, least squares mean difference in HbA_{1c} change between groups (GMet minus G) was -0.76% (95% CI: -1.10 to -0.42) demonstrating superiority of GMet to G, adjusted mean HbA_{1c} changes from baseline were -0.97% (-0.99 ± 0.94) and -0.22% (-0.20 ± 1.02) (Mean ± SD) in glimepiride/metformin and glimepiride groups, respectively; between-treatment difference -0.76% (95% CI -1.1 to -0.42; p<0.0001), showing statistical significance. Adjusted mean fasting plasma glucose level changes were -51.2mg/dl (-53.3 ± 53.9) and -39.8mg/dl (-37.6 ± 43.9) (Mean ± SD) from baseline, and the difference between two groups was -11.3mg/dl (95% CI -28.0 to 5.4; p=0.1807). 2 hr post prandial glucose levels changed by average -69.7 (-70.68 ± 88.8) for glimepiride/metformin group and -37.8 (-36.9 ± 86.6) mg/dl for glimepiride group with statistical difference of -31.9mg/dl (95% CI -61.6 to -2.3; p=0.0349). Differences of both HOMA-IR and β-cell changes between two groups were statistically non-significant (p=0.7409, 0.4835). The glimepiride/metformin group required lesser insulin amount than the glimepiride group (6.6 ± 9.9 and 12.5 ± 13.9 IU) (p=0.0204). Hypoglycemic events were experienced in 19 patients (39.6%) in glimepiride/metformin group and 20 patients (41.7%) in glimepiride group, and night time hypoglycemic events were experienced in 9 patients (18.8%) in both groups, but without any serious hypoglycemic adverse events.

Conclusion: In patients with type 2 diabetes poorly controlled on oral anti-diabetic therapy, insulin plus low-dose, fixed-combination therapy (glimepiride 1mg and metformin 500mg) or glimepiride 4mg therapy was effective in improving glycemic control, and both were well tolerated. The group with glimepiride/metformin was more effective in reducing HbA_{1c} and controlling after-meal hyperglycemic levels and this group required lesser insulin demand, without increasing hypoglycemic incidence.

Clinical Trial Registration Number: NCT00913367

Supported by: Handok Pharmaceuticals Co. Ltd/Sanofi-Aventis Korea

1039

Study of once-daily Levemir (SOLVE™) 3: safety of once-daily insulin detemir in patients with type 2 diabetes treated with oral antidiabetic therapy

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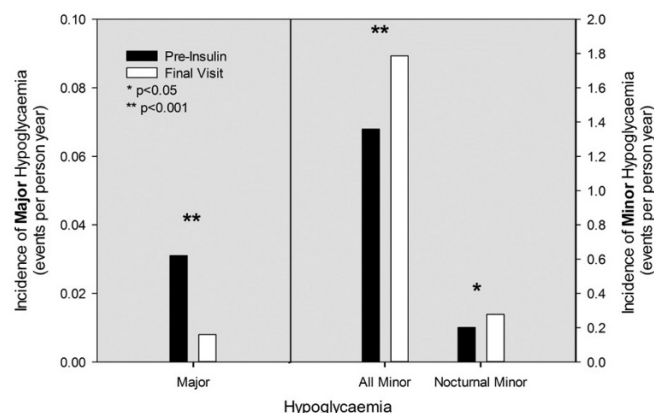
Background and aims: Concerns over the risks of hypoglycaemia, particularly major hypoglycaemia, remain an important barrier to diabetes treatment intensification and insulin initiation. The primary objective of the SOLVE study was to evaluate the incidence of serious adverse drug reactions, including major hypoglycaemic events during 24-weeks of treatment with once-daily insulin detemir in insulin naive patients with T2DM treated with one or more oral antidiabetic (OAD) drugs.

Materials and methods: This international cohort study was conducted in 10 countries. Data was collected at 3 routinely-scheduled clinic visits (baseline, 12-week, and 24-week visit). Hypoglycaemia episodes were defined as follows: major hypoglycaemia - event requiring third party assistance, minor hypoglycaemia - a daytime or nocturnal glucose measurement < 3.1 mmol/L +/- symptoms, and nocturnal hypoglycaemia - any minor episode occurring between bedtime and rising the next morning. Major hypoglycaemia was recorded as events recalled within the preceding 12 weeks (4 weeks in the UK), and minor hypoglycaemia as events recalled within the preceding 4 weeks (all countries).

Results: A total of 14,785 participants have been enrolled in the study. Of these, 10,786 participants have completed the 24 week study. Reasons for study withdrawal included loss to follow-up (n=17), discontinuation of OAD (n=20) or trial drug (n=38), incorrect trial drug regimen (n=50), addition of short acting insulin (n=81), adverse drug reaction (n=3) and other unspecified reasons (n=80). Participants included in the safety analysis were 53% male, age 62 ± 11 years, BMI 29.6 ± 5.3, and duration of diabetes of 10 ± 7 years, with a pre-insulin HbA_{1c} of 9.0 ± 1.6%. By the end of study, the HbA_{1c} had decreased to 7.7 ± 1.2%. The number of subjects experiencing major hypoglycaemia and/or a serious adverse drug reaction was n=21 (0.2%): comprising 15 patients with at least one episode of hypoglycaemia. The number of participants with any major hypoglycaemic event decreased from 44 (0.4%) pre-insulin to 11 (0.1%) by end of study (0.03 to <0.01 events per person year - see figure). The proportion with at least one episode of

minor hypoglycaemia increased from 3.6% pre-insulin to 6.2% by end of study (1.36 to 1.79 events per person year - see figure). The proportion with at least one episode of nocturnal hypoglycaemia also increased from 0.8% pre-insulin to 1.3% by end of study (0.20 to 0.28 events per person year).

Conclusion: The addition of once-daily insulin detemir to existing OAD regimens was safe and effective, and the severe adverse drug reactions were rare. The rates of hypoglycaemia, particularly major hypoglycaemia, were low despite clinically relevant reductions in HbA_{1c}. The distribution of hypoglycaemia with respect to OAD use are being analysed and will be presented at the meeting.



Clinical Trial Registration Number: NCT00825643

Supported by: Novo Nordisk A/S

1040

IDegAsp, a soluble insulin combination of ultra-long-acting insulin degludec and insulin aspart, in type 2 diabetes: comparison with biphasic insulin aspart 30

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Background and aims: Insulin degludec (IDeg) is a new-generation basal insulin that forms soluble multi-hexamers upon subcutaneous (s.c.) injection, resulting in an ultra-long and flat action profile. Insulin degludec/insulin aspart (IDegAsp) is a soluble co-formulation of IDeg (70%) and insulin aspart (IAsp, 30%) designed to provide mealtime and basal insulin coverage. In this phase 2, 16-week, open-label, treat-to-target trial, the safety and efficacy of IDegAsp were evaluated in insulin-naïve people with type 2 diabetes inadequately controlled on oral anti-diabetic drugs.

Materials and methods: Participants (mean: 60 years old; HbA_{1c} 8.5%; FPG 11.6 mmol/l; BMI 31.4 kg/m²) were randomised to twice-daily IDegAsp (n=61), BIAsp 30 (n=62) or an alternative formulation of IDegAsp (development discontinued, results not shown; n=59), all in combination with metformin (1500 or 2000 mg/day). Insulin was injected s.c. before both breakfast and the evening meal and titrated to a pre-breakfast and pre-dinner PG target of 4.0–6.0 mmol/l.

Results: Mean HbA_{1c} after 16 weeks (primary endpoint) was comparable for IDegAsp (6.7%) and BIAsp 30 (6.7%) (estimated treatment difference [ETD] IDegAsp-BIAsp 30 = -0.02% [-0.27; 0.24]). With IDegAsp, more subjects achieved HbA_{1c} <7.0% without confirmed hypoglycaemia (PG <3.1 mmol/l) in the last 4 weeks of treatment compared with BIAsp 30 (67% vs. 40% of subjects). Mean FPG at Week 16 was significantly lower for IDegAsp than BIAsp 30 (6.4 vs. 7.5 mmol/l; ETD: -0.99 mmol/l [-1.68; -0.29]). No severe hypoglycaemia was reported. The rate of confirmed hypoglycaemia was 58% lower for IDegAsp than BIAsp 30 (2.9 vs. 7.3 episodes/patient-year; estimated rate ratio: 0.42 [0.23; 0.75]). Nocturnal confirmed hypoglycaemia was less frequent for IDegAsp (7 episodes) than BIAsp 30 (20 episodes). The overall rate of adverse events was similar between insulins, the majority (>99%) were

mild or moderate in severity, and there was no treatment-specific pattern or clustering.

Conclusion: In this proof-of-concept trial IDegAsp was safe, well tolerated and provided comparable overall glycaemic control to BIAsp 30. IDegAsp was associated with a significantly lower FPG and a significantly lower rate of confirmed hypoglycaemia than BIAsp 30.

Clinical Trial Registration Number: NCT00613951

Supported by: Novo Nordisk

1041

Insulin degludec in a flexible daily dosing regimen provides similar glycaemic control without increasing rates of hypoglycaemia compared to dosing the same time daily in type 2 diabetes

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Background and aims: Current basal insulin preparations should be injected at a consistent time to ensure optimal biologic action. Patients have difficulty adhering to strict dosing schedules for many reasons. Insulin degludec (IDeg), which forms soluble multi-hexamers upon s.c. injection resulting in an ultra-long and consistent action profile, may enable more flexible dosing intervals as an alternative to the recommended strict dose timing of currently available insulins. In this registration trial for IDeg, we investigated whether once-daily administration of IDeg in a flexible dosing regimen (IDeg Flex) provides comparable glycaemic control as administration at the same time daily with the evening meal (IDeg OD).

Materials and methods: In this 26-week, open-label, treat-to-target trial, people with type 2 diabetes were randomised to IDeg Flex (n=229) and required to alternate the timing of insulin administration to morning and evening, in effect creating 8–40 h intervals between insulin doses, or to IDeg OD (n=228), where injections were given daily with the evening meal. Insulin was added to existing OAD therapy (if any) and titrated to FPG <5 mmol/l (90 mg/dl). Mean baseline characteristics such as age (56.2 vs. 56.5 yrs), HbA_{1c} (8.5 vs. 8.4%), diabetes duration (10.8 vs. 10.3 yrs) and BMI (29.3 vs. 29.4 kg/m²) were comparable between the IDeg Flex and IDeg OD groups, respectively.

Results: At 26 weeks, IDeg Flex and IDeg OD improved HbA_{1c} by 1.3 and 1.1 %-points, respectively (estimated treatment difference (ETD) IDeg Flex-IDeg OD: -0.13 %-points [95% CI: -0.29; 0.03]). Mean FPG was reduced from 9.0 to 5.8 mmol/l (IDeg Flex) and from 8.8 to 5.8 mmol/l (IDeg OD) (ETD: -0.05 mmol/l [-0.45; 0.35]). At Week 26, groups were similar in terms of fluctuations in 9-point self-measured plasma glucose (SMPG) (estimated treatment ratio (ETR) IDeg Flex/IDeg OD: 0.94 [0.86; 1.04]) and variation in pre-breakfast SMPG (ETR: 1.04 [0.94; 1.14]). Rates of confirmed hypoglycaemia (PG <3.1 mmol/l (56 mg/dl) or severe) were 3.6 episodes/patient-yr in both groups (estimated rate ratio (ERR) IDeg Flex/IDeg OD: 1.10 [0.79; 1.52]); the rate of nocturnal confirmed hypoglycaemia was 0.6 episodes/patient-yr in both groups (ERR: 1.18 [0.66; 2.12]). Severe hypoglycaemia was rare (2 episodes/group).

Conclusion: By using extreme dosing intervals of between 8 and 40 hours, the trial demonstrates that IDeg can be dosed flexibly at any time of the day and that changes in the injection time from day to day do not affect glycaemic control or risk of hypoglycaemia. The flexible dosing regimen made possible by IDeg will facilitate the integration of insulin therapy with daily activities and potentially improve adherence and acceptance of treatment.

Clinical Trial Registration Number: NCT01006291

Supported by: Novo Nordisk

1042

Differences in glycaemic reductions between insulin therapies according to body mass index: pooled results from multinational clinical trialsE. Wang¹, J. Lin², M.-P. Dain³, G. Dailey⁴¹sanofi-aventis, Bridgewater, USA, ²Novosys Health, Flemington, USA,³sanofi-aventis, Paris, France, ⁴Scripps Clinic and Research Foundation Division of Diabetes and Endocrinology, La Jolla, USA.

Background and aims: Insulin glulisine is an analog of human insulin designed for use as a rapid-acting insulin. This study used pooled data from 2 previously reported trials to compare the efficacy of twice daily glulisine with twice daily regular human insulin (RHI) in obese and non-obese patients. Patients had >6 months of continuous insulin therapy prior to study entry. HbA_{1c} results of the 2 studies showed noninferiority of glulisine to RHI ($P = 0.573$) and a small difference in favor of glulisine ($P = 0.003$), respectively.

Materials and methods: In the parent studies, 1666 patients with type 2 diabetes (mean HbA_{1c} 7.6%) were randomised and treated with glulisine + NPH ($n = 833$) or RHI + NPH ($n = 833$) for up to 26 weeks. Subjects were allowed to continue on the same dose of pre-study regimens of oral antidiabetic drug therapy (unless hypoglycaemia necessitated a dose change). Data for both trials were pooled then compared in patients with body mass index (BMI) \geq and < 30 kg/m². Additional analysis tested the impact of factors on change in HbA_{1c} from baseline to Month 6 using multivariable generalized linear model regression. Independent covariates included insulin type, age, gender, BMI, and baseline HbA_{1c}.

Results: This pooled analysis included 1666 patients with a mean (SD) age of 59.0 ± 9.7 years. 587 (35.2%) patients had a BMI < 30 (mean 26.9 ± 2.3 kg/m²) and 1079 (64.8%) had a BMI ≥ 30 (mean 36.1 ± 5.2). Female patients made up 40.2% and 42.2% of the glulisine + NPH and RHI + NPH groups, respectively, in the BMI < 30 cohort. In the BMI ≥ 30 cohort, 51.5% and 53.7% were female, respectively. Baseline HbA_{1c} was similar in the overall glulisine + NPH and RHI + NPH groups: 7.6 ± 0.9 % and 7.5 ± 0.9 %, respectively. Initiating with glulisine + NPH was associated with a greater reduction in HbA_{1c} than initiating with RHI + NPH in obese patients (glulisine + NPH: Δ HbA_{1c} = -0.45%; RHI + NPH: Δ HbA_{1c} = -0.33%, $P = 0.0219$). Reductions in HbA_{1c} among patients with BMI < 30 kg/m² were similar for glulisine + NPH (-0.28%) and RHI + NPH (-0.25%; $P = 0.7$). Other factors associated with significant reductions in HbA_{1c} in the overall pooled analysis included BMI ($P = 0.02$), age ($P < 0.001$), insulin treatment ($P = 0.04$) and baseline HbA_{1c} ($P < 0.001$) (Table). Serious hypoglycaemic episodes were experienced by 2.4% and 2.3% of glulisine + RHI patients, respectively, with BMI ≥ 30 ($P = 0.932$); and by 2.5% and 3.6% of glulisine + RHI patients, respectively with BMI < 30 ($P = 0.439$).

Conclusion: These results suggest that glulisine + NPH treatment in obese patients may result in greater HbA_{1c} reductions than RHI + NPH with comparable risk of hypoglycaemia. Other factors that were significantly associated with greater HbA_{1c} reduction in this analysis were older age, higher BMI and higher baseline HbA_{1c}.

Factors That Impacted Reduction of HbA_{1c}

Parameters	Impact on HbA _{1c} Reduction ^a	Confidence Interval	P-value
Glulisine vs RHI	-0.09	-0.003 to -0.17	0.04
Age (per year of increase)	-0.02	-0.02 to -0.01	< 0.001
Male vs. female	0.02	-0.07 to 0.11	0.7
BMI (per increase of 1 kg/m ²)	-0.01	-0.02 to -0.002	0.02
Baseline HbA _{1c} (per increase of 1 %)	-0.46	-0.51 to -0.41	< 0.001

^aNegative values represent greater HbA_{1c} reduction

Supported by: sanofi-aventis

PS 092 Insulin therapy in type 1 diabetes

1043

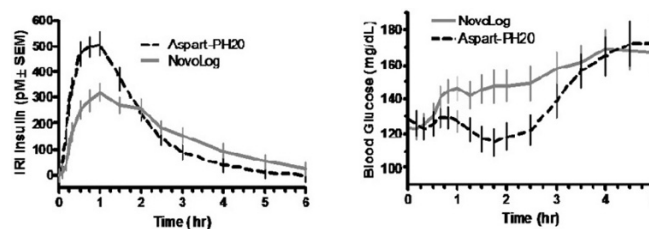
Accelerated absorption and reduced glycaemic variability in type 1 diabetes mellitus patients by human hyaluronidase-facilitated coinfusion of a prandial insulin analogueM. Hompesch¹, D.B. Muchmore², L. Morrow¹, D.E. Vaughn²¹Profil Institute for Clinical Research, Chula Vista, ²Halozyne Therapeutics, Inc., San Diego, USA.

Background and aims: To compare the pharmacokinetic (PK) and glucodynamic responses to insulin aspart delivered \pm human hyaluronidase by insulin pump for 72 hours in patients with type 1 diabetes mellitus (T1DM).

Materials and methods: An insulin aspart formulation (Aspart-PH20) with human hyaluronidase (rHuPH20) was compared to the commercial insulin aspart formulation (NovoLog[®]) for three days of diabetes treatment when delivered by continuous subcutaneous infusion in an inpatient setting. Data are available for 13 [9 male, 4 female; mean age $39.7 (\pm 9.7)$; mean BMI $26.2 (\pm 3.4)$] of planned 18 T1DM patients who regularly use insulin pumps. A euglycemic clamp experiment with a 0.15 U/kg bolus was conducted approximately $\frac{1}{2}$ day after infusion initiation; usual individual basal rate was continued during clamps and PK results are thus baseline-subtracted. Patients also received standardized solid evening meals (45-50% CHO, 18-22% protein, 30-34% fat) on each of three consecutive days and postprandial glycemic responses were measured by YSI.

Results: Aspart-PH20 was more rapidly absorbed than the commercial formulation leading to greater early exposure and action and reduced late exposure and action. Similar total exposure (63 ± 13 v. 55 ± 9 nM*min; 114%, $p = .096$) was shifted to earlier times post bolus infusion with a time to 50% exposure reduced from 119 ± 22 to 85 ± 20 minutes with rHuPH20 ($p = .0005$), resulting in 64% more insulin exposure in the 1st hour ($p < .0001$) and 58% less in beyond 4 hours ($p = .006$). Comparable insulin action, total glucose infused, (31.8 ± 13.5 v. 31.7 ± 11.0 mg/kg) was also accelerated with time to 50% glucose infused reduced from 154 ± 19 to 133 ± 19 minutes ($p = .007$), 20% more action in the first 2 hours ($p = .047$) and 37% less beyond 4 hours ($p = .008$). The accelerated insulin absorption and action led to reduced postprandial hyperglycemic excursion. The average two hour PPG for three consecutive dinners was reduced from 148 ± 34 to 118 ± 28 mg/dL for the formulation with rHuPH20 ($p = .009$) and total hyperglycaemic excursions ($AUC_{0-3hr} > 140$ mg/dL) was reduced 44% ($p = .02$). There were no significant differences in hypoglycemic risk, and the minimum PPG were similar with (83 ± 17) and without (88 ± 19 mg/dL) rHuPH20; $p = .46$. Both test articles were well tolerated.

Conclusion: Aspart-PH20 was absorbed more rapidly compared to the commercial aspart formulation, and this accelerated insulin exposure and action profile translated into superior control of postprandial blood glucose in patients with T1DM.



Clinical Trial Registration Number: NCT01275131

1044

Impact of the dawn phenomenon and the get-up phenomenon on insulin therapyG. Freckmann¹, A. Baumstark¹, S. Pleus¹, C. Haug¹, L.G. Krinkel², A. Buhr²¹Institute for Diabetes-Technology GmbH at the University of Ulm, Germany, ²Roche Diabetes Care AG, Burgdorf, Switzerland.

Background and aims: Many people with type 1 diabetes experience increased fasting blood glucose (BG) values after getting up. This increase is often caused by the dawn phenomenon but it may also be caused by another effect, the get-up phenomenon. During a study with closed-loop control we

observed the get-up phenomenon and compared it to the dawn phenomenon to analyze how this phenomenon should be considered in subcutaneous insulin infusion therapy.

Material and methods: 24 subjects with type 1 diabetes on continuous subcutaneous insulin infusion were enrolled for a study with overnight closed-loop control and visited the study site on three occasions. Each visit lasted three days. Blood sampling was performed once per hour between midnight and 06:00 A.M. and every 10 min after 06:30 A.M. The evaluation of the get-up phenomenon was performed on these BG values. Subjects were woken at 05:45 A.M. and had to get up until 06:00 A.M. The analysis was performed on all data gathered. A subgroup of patients who had at least three successful experiments was evaluated separately. An experiment was successful if there were no unusually high insulin delivery rates (more than 1.6 times the patient's basal rate), no hypo- or hyperglycemia interventions and no missing BG values and if no hourly basal rate was zero. We compared the BG difference before get-up (between 04:00 A.M. and 05:00 A.M. and between 05:00 A.M. and 06:00 A.M.) and after get-up (between 06:00 A.M. and 06:40 A.M.). **Results:** Results are given in the table.

Conclusion: The get-up phenomenon seemed to be more pronounced than the dawn phenomenon. Some patients may experience a significant BG increase after get-up. This phenomenon may be antagonized by increasing the basal rate in the morning or, if patients get up at different times of day, an additional bolus delivery. Further investigation is required to quantify the get-up phenomenon and to obtain information about patient groups most likely to be affected by this phenomenon.

BG difference in mg/dl/h (Mean \pm SD; [Range]) between:	All experiments (24 subjects, 140 nights)	Successful experiments (16 subjects, 70 nights)
- 04:00 A.M. and 05:00 A.M.	10.3 \pm 9.6; [-38.0 - 86.1]	6.6 \pm 6.5; [-15.5 - 30.0]
- 05:00 A.M. and 06:00 A.M.	8.1 \pm 10.3; [-29.2 - 65.0]	10.5 \pm 9.4; [-15.2 - 65.0]
- 06:00 A.M. and 06:40 A.M.	20.8 \pm 12.3 (*); [-38.6 - 84.0]	31.4 \pm 14.5 (*); [-15.0 - 84.0]

BG changes from 04:00 A.M. to 06:40 A.M. for all experiments and for successful experiments. The increase in BG after get-up is significantly larger than prior to get-up. (*) $p < 0.001$

Supported by: Roche Diabetes Care AG, Switzerland

1045

Basal-bolus therapy with insulin degludec improves long-term glycaemic control with less nocturnal hypoglycaemia compared with insulin glargine in type 1 diabetes: results of a 1-year trial

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Background and aims: This 1-year, open-label, treat-to-target trial compared the efficacy and safety of insulin degludec (IDeg), a new-generation basal insulin with an ultra-long and flat action profile, with insulin glargine (IGlar), both administered once-daily in combination with mealtime insulin aspart in basal-bolus therapy for type 1 diabetes management.

Materials and methods: The trial comprised 629 adults with type 1 diabetes who had previously been treated with any basal-bolus insulin therapy for at least 1 year (mean: age 43.0 years; diabetes duration 18.9 years; HbA_{1c} 7.7%). Participants were randomised in a 3:1 ratio to receive either IDeg or IGlar, respectively, and the basal insulin was titrated aiming to meet an FPG target <5 mmol/l.

Results: A similar proportion of participants completed the trial in the IDeg (86%) and IGlar (87%) groups. By the end of the trial, overall glycaemic control improved by 0.4 %-points with both IDeg and IGlar (estimated treatment difference (ETD) IDeg-IGlar: -0.01 %-points [95% CI: -0.14; 0.11]); the proportion of participants who achieved a target HbA_{1c} <7% was also comparable between the IDeg and IGlar groups (40% vs. 43%, respectively, $p = \text{NS}$). The observed reductions in mean FPG after 1 year, 1.27 mmol/l with IDeg and 1.39 mmol/l with IGlar, were similar (ETD: -0.33 mmol/l [95% CI: -1.03; 0.36] $p = \text{NS}$). The time taken to reach the titration target was significantly shorter in the IDeg group, compared with the IGlar group (median of 5 vs. 10 weeks, estimated hazard ratio: 1.37 [95% CI: 1.12; 1.67] $p = 0.002$). At the end of the study, mean daily total insulin doses were 0.75 U/kg and 0.82 U/kg for the IDeg and IGlar groups respectively, divided ~50:50 between the basal and bolus components for both groups. Rates of overall confirmed hypoglycaemia (PG < 3.1 mmol/l or severe episodes as per ADA definition) were comparable between IDeg and IGlar (42.5 vs. 40.2 episodes/patient-year; estimated rate

ratio (ERR) IDeg /IGlar: 1.07 [95% CI: 0.89; 1.28], $p = \text{NS}$). However, the rates of nocturnal confirmed hypoglycaemia were 25% lower with IDeg (4.4 vs. 5.9 episodes/patient-year; ERR: 0.75 [95% CI: 0.59; 0.96] $p = 0.021$). Overall rates of adverse events were similar between IDeg and IGlar, with no treatment-specific pattern or clustering.

Conclusion: Insulin degludec, given as basal-bolus treatment with insulin aspart in people with type 1 diabetes, improves long-term glycaemic control with a significantly lower rate of nocturnal hypoglycaemia compared with insulin glargine.

Clinical Trial Registration Number: NCT00982228

Supported by: Novo Nordisk

1046

Insulin degludec: two-fold longer half-life and a more consistent pharmacokinetic profile than insulin glargine

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Background and aims: Insulin degludec (IDeg) is a new-generation basal insulin that forms soluble multi-hexamers upon subcutaneous injection, resulting in an ultra-long and flat action profile. We describe the pharmacokinetic (PK) properties of IDeg in comparison to those of insulin glargine (IGlar) under steady-state (SS) conditions in people with type 1 diabetes.

Materials and methods: This was a randomised, double-blind, two-period, crossover study. Sixty-six people with type 1 diabetes (55 males/11 females, mean age 37 years, BMI 24.9 kg/m², HbA_{1c} 8.1%) received one of three fixed doses (0.4, 0.6 or 0.8 U/kg) of IDeg and IGlar once daily for 8 days with 7-21 days wash-out between treatments. A euglycaemic glucose clamp was performed on treatment Day 8 and PK samples were taken throughout each treatment period and for 120 h after the last dose.

Results: IDeg showed stable PK concentrations under steady-state conditions that showed minimal fluctuations and that increased proportionally with increasing dose. The serum exposure to IDeg was equally distributed between the first and the second 12 hours post-dosing (indicated by a ratio between AUC_{0-12h,SS} and AUC_{total,SS} of 0.5) whereas IGlar showed a higher exposure during the first 12 hours (AUC_{0-12h,SS} / AUC_{total,SS} = 0.6). Likewise, the cumulated AUC below and above the average glucose infusion rate (AUC_{GIR,SS}) was considerably lower for all doses of IDeg (0.25, 0.37, 0.38 mg/kg/min) than with IGlar (0.39, 0.54 and 0.73 mg/kg/min). IDeg was detectable in the serum for at least 120 h following the final dose, whereas, for most subjects, IGlar fell below the lower limit of quantification after 36-48 h post dosing. Mean terminal half-life was twice as long for IDeg than IGlar (25.4 vs. 12.5 h). Both insulin preparations were well tolerated and no safety concerns were identified.

Conclusion: IDeg has a half-life that is twice as long as IGlar, resulting in a more evenly distributed and stable pharmacokinetic profile for IDeg at steady state in people with type 1 diabetes.

Clinical Trial Registration Number: NCT01114542

Supported by: Novo Nordisk

1047

Ultra-long pharmacokinetic properties of insulin degludec in adults with type 1 diabetes is preserved in children and adolescents after single-dose administration

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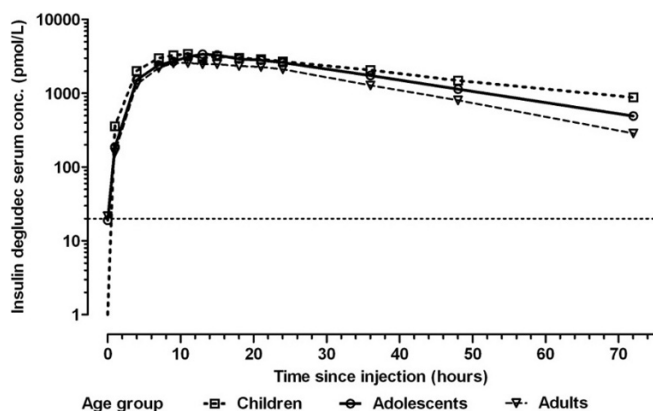
Background and aims: The incidence of type 1 diabetes in children/adolescents is growing with a predicted increase of 70% in prevalent cases ≤ 15 years in Europe. Management of type 1 diabetes in children and adolescents requires continuous monitoring and frequent adjustment of basal and bolus insulin. Insulin degludec (IDeg) is a neutral soluble ultra-long-acting new-generation basal insulin with duration of action of more than 42h in adults, allowing flexible dosing at any time of the day with the possibility to change injection time from dose to dose. The primary objective of this trial was to investigate how the pharmacokinetic (PK) properties of IDeg in children and adolescents compare with those in adults after a single dose.

Materials and methods: This was a randomised, single-centre, double-blind, single dose, cross-over trial with IDeg and insulin glargine. 12 children, 13

adolescents and 12 adults (mean age 10.3 ± 1.1 , 14.3 ± 1.6 , and 25.6 ± 1.9 y; BMI 18.6 ± 1.9 , 21.5 ± 2.0 , and 25.3 ± 2.9 kg/m²; mean duration of diabetes 5.1 ± 2.4 , 5.9 ± 4.1 , and 13.8 ± 8.3 y; HbA_{1c} 7.7 ± 0.8 , 7.7 ± 0.5 , and 7.6 ± 1.0 %) completed the trial and were included in the PK evaluation. IDeg (100 U/ml) was administered as a single s.c. dose (0.4 U/kg). Blood samples for PK analyses were drawn over the first 48h, and again at 72h post-dosing.

Results: Duration of exposure to IDeg was ultra-long in children, adolescents and adults after a single dose (see mean profiles). IDeg was detected 72h after administration for all subjects. Total exposure to IDeg ($AUC_{IDeg,0-\infty}$) tended to be greater in children and adolescents than in adults based on pairwise comparisons (children/adults 1.48 [95% CI: 0.98;2.24], adolescents/adults 1.33 [95% CI: 1.08;1.64]). No statistically significant differences in maximum IDeg concentration ($C_{max,IDeg}$) were shown between children and adults (1.20 [95% CI: 0.90;1.60]) or adolescents and adults (1.23 [95% CI: 1.00;1.51]). There were no differences between age groups regarding the number of hypoglycaemic episodes, and no episodes of severe hypoglycaemia. No clinically significant changes in laboratory parameters, vital signs or physical examination were observed.

Conclusion: The ultra-long-acting properties of IDeg in adults are preserved in children/adolescents. Extent of exposure after a single fixed dose tends to be greater in children/adolescents than adults; as with other insulin products, IDeg should always be titrated according to individual requirements. IDeg is well tolerated and no safety issues are identified in this trial. Thus, paediatric studies investigating the potential clinical benefits of this ultra-long-acting basal insulin with unprecedented duration of action appear warranted.



Dashed line represents the lower limit of quantification

Clinical Trial Registration Number: NCT01030926

Supported by: Novo Nordisk

1048

Does insulin detemir alter satiety or caloric intake to control weight?

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Background and aims: Insulin detemir is a long-acting insulin analog that is weight-neutral compared with other long-acting insulins in patients with type 1 diabetes, but the mechanism for this observation is unknown. One mechanism may be an effect of detemir to enhance satiety. We hypothesized that type 1 diabetes patients would eat fewer calories when presented with a standardized buffet meal following a 24 hour fast when using insulin detemir as compared to insulin glargine.

Materials and methods: Ten subjects with C-peptide negative type 1 diabetes participated in a randomized, double-blind crossover study in which they received equivalent doses of either insulin detemir or glargine twice daily for at least 3 weeks. They were then admitted to the UNM Clinical Research Unit for a 24 hour fast, after which they were allowed to eat to satiety from a standardized, 10,000 calorie buffet without provision of rapid acting insulin. Measurement of caloric consumption following the fast, body composition by bioelectrical impedance, hunger assessment, and indirect calorimetry were performed. The serum satiety factors leptin, ghrelin and PYY were assessed at baseline, after the 24 hour fast, and after ingestion of the test meal.

Results: Subjects were aged 35 ± 11 years, had diabetes for 18 ± 11 years, had HbA_{1c} levels of 8 ± 1 % and BMI of 30 ± 8 kg/m². Long acting insulin doses

were 37 ± 26 units/day. Home glucose values did not differ during treatment between the insulins during the study (187 ± 254 detemir vs. 172 ± 269 mg/dl glargine; $p=0.21$), but short-acting insulin doses were higher on detemir (15 ± 10 vs. 13 ± 8 units/day; $p<0.001$). Hunger scores (68 ± 25 vs. 73 ± 24 ; $p=0.58$) and total energy ingested following the 24 hour fast (1418 ± 636 vs. 1357 ± 576 kcal; $p=0.63$) did not differ between insulin detemir and insulin glargine, respectively. Resting Energy Expenditure also did not differ (1405 ± 398 vs. 1457 ± 536 kcal/d; $p=0.63$). Results of satiety factors are shown in the table and did not differ significantly.

Conclusion: The weight-neutrality of insulin detemir in type 1 diabetes is not attributable to reduced caloric intake, and systemic satiety factors are unaffected by choice of long acting insulin.

Satiety Factors Before and After Fasting and Feeding

	Leptin (ng/ml)			PYY (pg/ml)			Ghrelin (pg/ml)		
	Baseline	Fasted	Fed	Baseline	Fasted	Fed	Baseline	Fasted	Fed
Glargine	19.8±18.4	18.7±18.6	17.2±16.4	90±37	62±19	96±42	130±102	91±60	92±25
Detemir	15.1±13.5	13.0±11.9	14.2±13.7	99±66	83±85	89±31	129±70	139±151	115±82

Clinical Trial Registration Number: NCT00659165

Supported by: UNM Clinical and Translational Research Center

1049

Multi-hexamer formation is the underlying basis for the ultra-long glucose-lowering effect of insulin degludec

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Background and aims: Insulin Degludec (IDeg) is a new-generation basal insulin that has been shown in clinical studies to have a flat and stable action profile and a lower risk of hypoglycaemia compared to other basal insulins. A continuous and sustained release of IDeg monomers into the circulation from a depot of soluble multi-hexamers that form after subcutaneous (s.c.) injection is considered to be the underlying mechanism behind the distinct characteristics of IDeg. Here we use transmission electron microscopy (TEM) to verify the formation of IDeg multi-hexamers under conditions mimicking the s.c. injection site, and investigate the pharmacodynamic consequences of IDeg multi-hexamer formation through use of the euglycaemic glucose clamp in people with type 1 diabetes.

Materials and methods: Transmission electron microscopy was used to visualise IDeg multi-hexamers. Samples of IDeg at a concentration of 2.5 mg/ml in a preparation containing five zinc ions per insulin hexamer were placed on formvar coated copper grids, stained with a 2.5 % (m/V) uranyl acetate solution and examined with a FEI Morgagni 268 electron microscope, operating at 80 kV. The duration of the glucose-lowering effect of IDeg was investigated in 42-h euglycaemic glucose clamps (Biostat; clamp blood glucose (BG) level: 5.5 mmol/l) conducted after 8 days of once-daily administration of IDeg (0.4, 0.6 or 0.8 U/kg) to people with type 1 diabetes.

Results: Under conditions mimicking the s.c. interstitial fluid, TEM revealed elongated structures with a uniform width (6.3 ± 0.9 nm) that was consistent with the expected width of insulin hexamers. No such structures were visible under conditions corresponding to the pharmaceutical IDeg formulation, indicating that multi-hexamers only form after s.c. injection. Addition of EDTA to chelate zinc ions disrupted the multi-hexameric structures. This demonstrates that multi-hexamer formation is reversible, and suggests that the gradual release of zinc ions from s.c. multi-hexamers leads to the release of IDeg monomers for absorption. In the euglycaemic glucose clamp experiments, the glucose-lowering effect of IDeg extended beyond 42 hours at all three doses. End of action (BG >8.3 mmol/l) did not occur within the 42-h clamp period for any of the subjects dosed with 0.6 or 0.8 U/kg IDeg, and only for three out of the 21 subjects dosed with 0.4 U/kg IDeg. Moreover, mean BG profiles measured over the 42-h clamp remained almost horizontal for the 0.6 and 0.8 U/kg dose groups showing that BG was controlled throughout.

Conclusion: The formation of soluble IDeg multi-hexamers at the subcutaneous injection site gives rise to an ultra-long glucose-lowering effect that extends beyond 42 h at clinically relevant doses in people with type 1 diabetes.

Clinical Trial Registration Number: NCT01114542

Supported by: Novo Nordisk

1050

IDegAsp, a soluble insulin combination of ultra-long-acting insulin degludec and insulin aspart, used once daily in basal-bolus treatment with insulin aspart in type 1 diabetes

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Background and aims: Insulin degludec (IDeg) is a new-generation basal insulin that forms soluble multi-hexamers upon subcutaneous (s.c.) injection, resulting in an ultra-long and flat action profile. Insulin degludec/insulin aspart (IDegAsp) is a soluble co-formulation of IDeg (70%) and insulin aspart (IAsp; 30%) designed to provide mealtime and basal insulin coverage. This phase 3, 26-week, open-label, treat-to-target trial investigated the efficacy and safety of IDegAsp in people with type 1 diabetes inadequately controlled on any insulin.

Materials and methods: 548 people with type 1 diabetes and inadequately controlled HbA_{1c} (mean: age 41 years; HbA_{1c} 8.3%; FPG 10.5 mmol/l) on any insulin-based regimen were randomised 2:1 to receive IDegAsp or insulin detemir (detemir). IDegAsp was administered once-daily and could be given at any meal, with IAsp at remaining meals. Detemir was administered according to label with IAsp at all meals.

Results: Similar proportions of participants completed the trial (87% for IDegAsp and 86% for detemir). IDegAsp and detemir treatment resulted in similar improvements in HbA_{1c} (0.73 %-point reduction for IDegAsp vs. 0.68 %-point reduction for detemir; estimated treatment difference (ETD) IDegAsp-detemir: -0.05 %-point [95% CI: -0.18; 0.08]). FPG was reduced by 1.6 mmol/l with IDegAsp and by 2.4 mmol/l with detemir; ETD IDegAsp-detemir: 0.23 mmol/l [-0.46; 0.91] p=0.52). Confirmed hypoglycaemia (PG <3.1 mmol/l or severe) was reported for ~94% of subjects in both groups; rates were similar (39 vs. 44 episodes/patient-year; estimated rate ratio (ERR) IDegAsp/IDet: 0.91 [95% CI: 0.76; 1.09] p=0.27). The rate of nocturnal confirmed hypoglycaemia (confirmed hypoglycaemia occurring between 00:01-05:59 h) was 37% lower with IDegAsp (3.7 vs. 5.7 episodes/patient-year; ERR: 0.63 [95% CI: 0.49; 0.81] p=0.0003). Mean total daily insulin doses were similar in both groups by the end of the study (0.86 U/kg vs. 1.00 U/kg, for IDegAsp and detemir groups, respectively). After 26 weeks, mean body weight had increased from 76.5 to 78.9 kg (IDegAsp) vs. 76.1 to 77.5 kg (detemir); there was less weight gain in the detemir group compared with the IDegAsp group (ETD: IDegAsp-detemir: 1.0 kg [0.38; 1.69] p=0.0021). Overall rates of adverse events were similar between groups with no treatment-specific pattern or clustering.

Conclusion: IDegAsp dosed once daily at any meal with IAsp at the remaining meals provided similar glycaemic control to detemir with mealtime IAsp. The IDegAsp regimen was associated with significantly less nocturnal hypoglycaemia, and has the added convenience of fewer daily injections than conventional basal-bolus therapy.

Clinical Trial Registration Number: NCT00978627

Supported by: Novo Nordisk

Materials and methods: Participants (n=33) (mean age of 40 ± 10 years, diabetes duration of 20 ± 10 years, HbA_{1c} 7.3 ± 0.8% and C-peptide of <0.3 nmol/l) each underwent 8-day treatment periods with 0.4 U/kg U100 and U200 given once daily with insulin aspart at mealtimes. On Day 8, participants fasted and a glucose clamp procedure was performed with euglycaemia (5.5 mmol/l) established and maintained using a variable intravenous glucose infusion over 5 hours, whereupon IDeg (0.4 U/kg U100 or U200) was injected at the usual dosing time and the clamp maintained for 26 hours. The primary endpoint was AUC_{GIR,total,SS}.

Results: Comparable glucose infusion rates (GIR) were observed for U100 and U200 (AUC_{GIR} [mg/kg]: 2255 vs. 2123) and the mean ratio of U200/U100 for the primary endpoint (AUC_{GIR,total,SS}) was 0.94 [95% CI: 0.86; 1.03]. A post-hoc analysis showed bioequivalence between U100 and U200, as the 90% CIs of the ratios for AUC_{IDeg,total,SS} and C_{max,IDeg,SS} were within the interval 0.80-1.25 (AUC_{IDeg,total,SS} U200/U100 ratio 0.99 [0.91; 1.07] and C_{max,IDeg,SS} U200/U100 ratio 0.93 [0.84; 1.02]). Both formulations were well tolerated, and there were no marked differences in distribution of adverse events or hypoglycaemic events.

Conclusion: Insulin degludec U100 and U200 formulations are bioequivalent and have similar pharmacodynamic profiles at steady state, suggesting that they can be used interchangeably in clinical practice.

Clinical Trial Registration Number: NCT01076634

Supported by: Novo Nordisk

1051

Ultra-long-acting insulin degludec: bio-equivalence and similar pharmacodynamics shown for two different formulations (U100 and U200)

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Background and aims: Insulin degludec (IDeg) is a new-generation basal insulin that forms soluble multi-hexamers upon subcutaneous injection resulting in a depot from which IDeg is continuously and slowly absorbed to provide an ultra-long action profile. Two different formulations, 100 U/ml (U100) and 200 U/ml (U200), have been developed, the aim of the latter being to enable higher doses to be administered in smaller injection volumes. A double-blind, crossover, randomised study was conducted to characterise the pharmacodynamic response and pharmacokinetic exposure to U100 and U200 under steady-state (SS) conditions in people with type 1 diabetes.

PS 093 New aspects of insulin therapy

1052

Durability of glycaemic control with insulin lispro mix 25 vs glargine for older patients with type 2 diabetes

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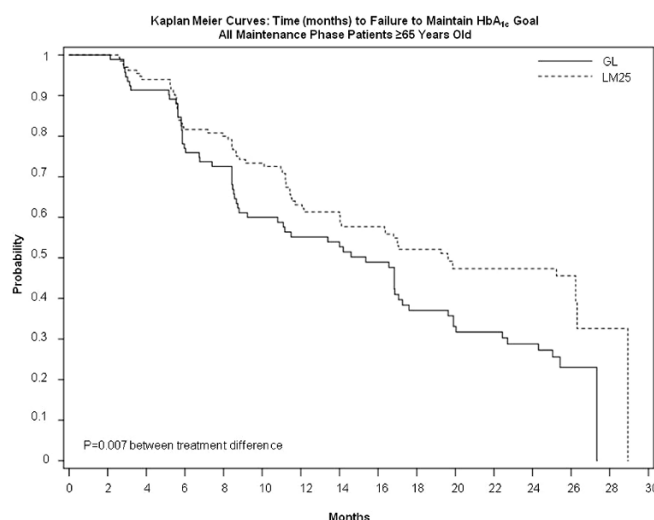
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Background and aims: Few clinical trials have evaluated long-term durability of glycaemic control in older patients. This post-hoc analysis compared durability of glycaemic control of 2 starter insulin regimens in older patients (≥ 65 years of age) in the DURABLE Trial maintenance phase: twice-daily insulin lispro mix 25 (LM25; 25% insulin lispro, 75% insulin lispro protamine suspension) vs once-daily insulin glargine (GL), added to ≥ 2 oral antihyperglycaemic medications in insulin-naïve patients with type 2 diabetes.

Materials and methods: In the 24-week initiation phase, patients were randomised to LM25 vs GL, after which those with $HbA_{1c} \leq 7.0\%$ entered a 24-month maintenance phase. Among the 892 patients in the maintenance phase, 224 were older (≥ 65 years of age) (LM25, $n=133$; GL, $n=91$). The present analysis primarily compared duration of maintaining HbA_{1c} goal ($HbA_{1c} \leq 7.0\%$, or $HbA_{1c} > 7.0\%$ with $< 0.4\%$ increase from last $HbA_{1c} \leq 7.0\%$) for LM25 vs GL among older patients. Analyses were also conducted for older patients achieving 24-week HbA_{1c} targets $\leq 6.5\%$ (LM25, $n=72$; GL, $n=39$).

Results: Baseline characteristics were similar for LM25 vs GL. The mean age was 69.5 years, baseline weight 89.5 kg, BMI 32.0 kg/m², duration of diabetes 11.8 years, and female 40.2%. Median time of maintaining HbA_{1c} goal (see Figure) was significantly longer in LM25 (19.6 months; 95%CI=14.0, 26.3) vs GL (15.4 months; 95%CI=9.2, 17.3; $p=0.007$) and more patients in LM25 maintained HbA_{1c} goal vs GL (49.2% vs 30.4%; $p=0.003$). HbA_{1c} reduction from baseline was significantly greater in LM25 vs GL ($-1.56 \pm 0.10\%$ vs $-1.24 \pm 0.11\%$; $p=0.003$), including older patients with $HbA_{1c} \leq 6.5\%$ ($-1.61 \pm 0.14\%$ vs $-1.34 \pm 0.12\%$; $p=0.049$). Endpoint fasting blood glucose was similar in LM25 vs GL, but post-meal glucose was significantly lower in LM25 vs GL (8.82 ± 0.19 mmol/L vs 9.53 ± 0.25 mmol/L; $p=0.017$), including older patients with $HbA_{1c} \leq 6.5\%$ (8.33 ± 0.20 mmol/L vs 9.31 ± 0.41 mmol/L; $p=0.016$). Overall hypoglycaemia rates and severe hypoglycaemia incidence were similar in LM25 vs GL, including older patients with $HbA_{1c} \leq 6.5\%$. Weight gain was significantly greater in LM25 vs GL (5.47 ± 0.49 kg vs 3.10 ± 0.53 kg; $p=0.001$), but was similar in older patients with $HbA_{1c} \leq 6.5\%$. Daily insulin doses were significantly higher in LM25 vs GL (0.41 ± 0.02 IU/kg/day vs 0.32 ± 0.02 IU/kg/day; $p<0.001$); however, were similar for LM25 vs GL in older patients with $HbA_{1c} \leq 6.5\%$.

Conclusion: In the older population, LM25 resulted in longer durability of glycaemic control, and a greater number of patients maintaining HbA_{1c} goal vs GL. LM25 was associated with more weight gain and modestly higher daily insulin doses; hypoglycaemia rates and incidence of severe hypoglycaemia were similar for LM25 vs GL. Older patients with $HbA_{1c} \leq 6.5\%$ had similar results, including no increase in hypoglycaemia with LM25 vs GL.



Clinical Trial Registration Number: NCT00279201

Supported by: Lilly USA, LLC

1053

Factors associated with HbA_{1c} reduction in Asian patients with T2DM: an analysis of the FINE Asia registry

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Background and aims: Asian patients with type 2 diabetes mellitus (T2DM) who were uncontrolled on oral antihyperglycemic drugs (OADs) were recruited from 11 countries in the FINE Asia registry. The study included 2921 (50.3% female) patients, with a mean (SD) age of 56.4 (11.2) years and a T2DM duration of 9.3 (6.5) years. A total of 2196 (75.2%) patients were prescribed insulin glargine, 637 (21.8%) NPH insulin, and 75 (2.6%) insulin detemir. After 6 months of basal insulin therapy with or without concomitant OADs, there was marked improvement in glycaemic control. Overall, HbA_{1c} decreased from 9.8 (1.6) to 7.7 (1.4) % at Month 6. This analysis aimed to identify factors responsible for the HbA_{1c} change.

Materials and methods: Multivariate generalized linear model (GLM) regression was used where independent covariates included age, gender, initiating insulin type and other demographic and clinical characteristics related to diabetes management; $p < 0.05$ was considered statistically significant. In calculating p values, the statistical model factored the impact of different sample sizes.

Results: There were 2679 patients with HbA_{1c} values at baseline and Month 6 who were included in the analysis. Initiating insulin with detemir ($n = 61$) vs glargine ($n = 2016$) was associated with less reduction in HbA_{1c} ($\Delta = -0.50$; 95% confidence interval [CI]: -0.17, -0.83; $P = 0.003$), as was initiating with NPH ($n = 589$) vs glargine ($\Delta = -0.36$; 95% CI: -0.23, -0.49; $P < 0.001$). Older age was associated with greater reduction in HbA_{1c} ($\Delta = 0.008$; 95% CI: 0.003, 0.013; $P = 0.002$) as was a higher HbA_{1c} value at baseline ($\Delta = 0.8$; 95% CI: 0.77, 0.83; $P < 0.001$). A longer history of previous OAD usage was associated with less reduction in HbA_{1c} ($\Delta = -0.026$; 95% CI: -0.052, -0.0001; $P = 0.049$) as was female gender ($\Delta = -0.19$; 95% CI: -0.06, -0.32; $P = 0.005$). There was no significant association of HbA_{1c} reduction with baseline body weight, fasting blood glucose, blood pressure or insulin dose or with duration of diabetes.

Conclusion: Among patients with T2DM in Asia, the following factors were associated with greater HbA_{1c} reduction: initiation with glargine vs NPH or detemir, older age, and higher HbA_{1c} at baseline. Identification of these and other factors may support better glycaemic control.

Supported by: sanofi-aventis

1054

Pharmacodynamics of basal insulins NPH, glargine and detemir in type 2 diabetes mellitus: effects of adiposity

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Background and aims: The role of adiposity of Type 2 diabetes (T2DM) on pharmacodynamics (PD) of basal insulins NPH, detemir (Det) and glargine (Gla) is not known.

Materials and methods: To examine the question, we studied the relationship between PD and body mass index (BMI), in 18 subjects with Type 2 diabetes (age 60 ± 7 yrs, BMI 29.1 ± 3.2 kg/m², A1C $7.5 \pm 0.6\%$, treatment insulin+oral hypoglycemic agents [mean \pm SD]). PD results (glucose infusion rate over 32h [GIR_{0-32h} , AUC_{0-32h}]) were generated by a randomized, single-blind, cross-over study using the euglycemic clamp (100 mg/dl) for 32 h after s.c. injection of 0.4 U/kg at 22.00 h of either NPH or Gla or Det, after 1-week treatment with each insulin, and analyzed according to BMI.

Results: Based on BMI status (<29 and >29 kg/m², i.e. below and above the median BMI of the overall group), GIR was greater in people with BMI <29 kg/m² compared to those with BMI >29 kg/m² although statistical significance was achieved only with Det (1564 ± 649 and 598 ± 604 mg/Kg, respectively, $p=0.03$) and not with NPH (1282 ± 532 and 1058 ± 859 mg/Kg) and glargine (1668 ± 807 and 1408 ± 563 mg/Kg), (both $p>0.2$). A multiple regression analysis after correcting for age and duration of diabetes revealed a statistically significant inverse correlation between BMI and GIR only with Det ($r=-0.68$, $p=0.003$), but not with Gla ($r=-0.41$, $p=0.11$) and NPH ($r=-0.37$, $p=0.15$). In addition, a positive correlation was found between BMI and endogenous glucose production (EGP) with NPH and Det ($r=0.66$, $p=0.005$ and $r=0.62$, $p=0.011$, respectively) but not with Gla ($r=0.35$, $p=0.17$). As expected, PD of basal insulins in T2DM is inversely correlated with adiposity, likely because of greater insulin resistance in BMI >29 kg/m².

Conclusion: Among the three insulins examined, Det exhibits the lowest PD action and weakest effect in restraining EGP as adiposity increases, as compared to NPH and Gla. PD of Gla (mediated by greater suppression of EGP) is less affected by adiposity as compared to NPH and to greater extent, Det. These findings may explain, at least in part, the need for higher doses of Det as compared to Gla in clinical trials in obese T2DM when the two basal insulins are titrated to same target A1C.

1055

Ultra-long-acting insulin degludec has a flat and stable glucose-lowering effect

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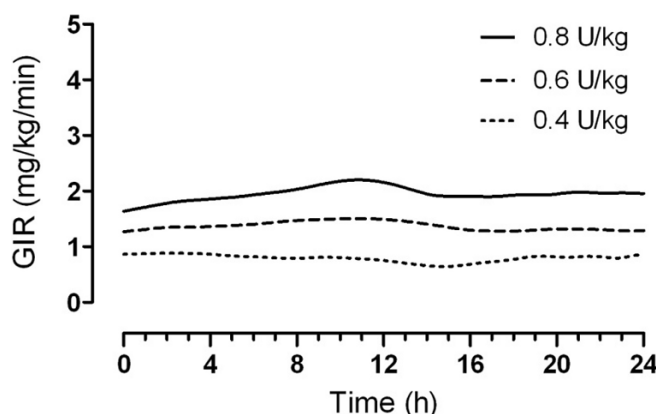
Background and aims: Insulin degludec (IDeg) is a new-generation, ultra-long-acting basal insulin that forms soluble multi-hexamers upon subcutaneous injection, resulting in a depot from which IDeg is continuously and slowly absorbed into the circulation. In this trial we investigated the dose-response relationship of IDeg at steady state (SS) in people with type 2 diabetes.

Materials and methods: In this double-blind, two-period, cross-over trial, the dose-response relationship of three doses of IDeg (0.4, 0.6 and 0.8 U/kg) was evaluated at steady state. Participants (insulin-treated people with type 2 diabetes without concomitant oral anti-diabetic agents, $n=49$; mean: age, 58.7 years; BMI, 29.6 kg/m²; HbA_{1c}, 7.6%; duration of diabetes, 14.1 years) were given IDeg once-daily for 6 days, with a washout period of 13–21 days between treatments (starting after the last dosing on Day 6). Following dosing on Day 6, subjects underwent a euglycaemic glucose clamp (Biostat; clamp blood glucose level: 5 mmol/l). Pharmacokinetic samples were taken up to 120 h after the last injection of IDeg.

Results: For all dose levels, mean 24-h glucose infusion rate (GIR) profiles were flat and stable (Figure 1). Total glucose-lowering effect ($AUC_{GIR, total, SS}$) increased linearly with increasing dose. Over 24 h, the glucose-lowering effect of IDeg was evenly distributed between the first and second 12 h for all three dose levels ($AUC_{GIR, 0-12h, SS} / AUC_{GIR, total, SS} = 0.5$). The blood glucose levels of all participants stayed very close to the clamp level until the end of the experiment (mean blood glucose levels in the last 10 min of a 24-h dosing interval were 5.0–5.1 mmol/l for all IDeg doses). The terminal half-life estimated across the three dose levels after the last dose was 25.1 hours. IDeg was well tolerated and no safety concerns were identified.

Conclusion: IDeg has a flat and stable blood glucose-lowering effect, and a duration of action beyond 24 h in people with type 2 diabetes.

Figure 1: Mean 24-h glucose infusion rate profiles at steady state



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Metabolism of insulin glargine after subcutaneous injection of therapeutic dose in type 2 diabetes mellitus

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Background and aims: After subcutaneous (sc) injection *in vivo*, the long-acting insulin analog glargine (GLA) undergoes a sequential cleavage of the carboxy terminus of the B-chain forming metabolites M1 and M2 that lack the di-arginine (M1 after removal of the two arginines, M2 with additional deamination of threonine at position B30). M1 and M2 have the same metabolic properties as human insulin (HI) and do not differ from HI in affinity for IGF-IR. Currently, there are no data about dynamics and relative percentage of plasma M1 and M2 vs GLA after sc dose in patients with type 2 diabetes (T2DM).

Materials and methods: GLA, M1, and M2 plasma concentrations were determined from samples obtained from a single-center, 32h euglycemic glucose clamp study, where 18 subjects with T2DM received a single sc dose of 0.4 U/kg GLA after one week of daily administration. Data from 9 subjects and 31h glucose clamp, are here reported (mean \pm SD: BMI 29.3 ± 3 kg/m²; A1C $7.4 \pm 0.0\%$, diabetes duration 13 ± 10 yrs). GLA, M1, and M2 were extracted using immunoaffinity columns and quantified by a specific liquid chromatography tandem mass spectrometry assay, without cross-reactivity to endogenous human or other insulins. The limit of quantitation (LOQ) was 0.2 ng/ml (~ 33 pmol/l).

Results: GLA was detected in 5 of the 9 subjects and only at a few time points; M2 was not detected at all, whereas M1 was detected in all subjects. M1, but not GLA, was detected at baseline (median: 44 pmol/l; 25th to 75th percentile: 19 to 131 pmol/l), likely reflecting GLA injected 24h before. In fact, 24h post-injection values were similar to baseline values (60; 52 to 118 pmol/l). The median GLA PK-AUC₀₋₃₁ was 171 pmol·h/l (0 to 314) and M1 PK-AUC₀₋₃₁ was 2166 pmol·h/l (1622 to 4955). GLA C_{max} was 40 pmol/l (0 to 48) and M1 C_{max} was 129 pmol/l (75 to 207).

Conclusion: After sc injection of a therapeutic dose, GLA is minimally detectable in blood with low peak concentration and up to only 9 hours (3.5 to 14.5), whereas its metabolite M1 accounts for most ($\sim 90\%$) of the plasma insulin concentration up to 31 h. Additional studies using a higher dose of GLA might provide further insights into the metabolism of GLA in T2DM

Supported by: Sanofi-Aventis

1057

Insulin glargine as well as metformin therapy improves myocardial function in patients with coronary artery disease and impaired glucose tolerance or early type 2 diabetes

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Background and aims: Patients with coronary artery disease (CAD) have a 40–60% probability of an impaired glucose metabolism. The risk for cardiovascular disease is already increased in patients with pre-diabetes, e.g. impaired glucose tolerance (IGT) and this risk increases further with type 2 diabetes (T2D). Treatment algorithms for patients with prediabetes do not exist. Accordingly, we created this hypothesis creating, randomized controlled trial about effects of a medical treatment in patients with IGT or early T2D with CAD focusing on cardiovascular function and on postprandial (pp) effects of this therapy.

Materials and methods: Inclusion criteria were CAD with IGT or early diabetes (either diet alone or a maximum of one oral antihyperglycemic agent). We recruited 28 patients, age 66 ± 10 years (IGT $n=12$, diabetes $n=16$ with a duration of 3 ± 3 years). They were randomized to metformin (Met), 2000 mg daily or insulin glargine (IG) to be titrated to a fasting glucose ≤ 110 mg/dl. Blood tests and echocardiography were done fasting and 2 hours after a standardized carbohydrate (48g) meal at baseline and 4, 12 and 24 weeks. Systolic (S') and diastolic cardiac function (E') were assessed by pulsed tissue Doppler. Age, HOMA, concomitant medication and the history of CAD were comparable between the groups.

Results: Patients on IG ($n=13$, mean daily dose 17 IU) reported no hypoglycaemic events or other side effects. Two patients on Met ($n=15$, mean daily dose 1518 mg) reported mild abdominal side effects. S' remained unchanged in both groups, but E' improved significantly in IG by $12 \pm 18\%$ ($p<0.03$) and in Met by $7 \pm 12\%$ ($p<0.05$) in the fasting state and also pp ($p<0.05$ and <0.02 respectively). Systolic blood pressure decreased in IG and Met (by 11 ± 14 , $p<0.02$ and by 15 ± 18 mmHg, $p<0.01$) as did diastolic blood pressure ($p<0.04$ and <0.03). The intergroup difference between the observed reduction of intima media thickness (IMT) in IG vs. the observed increase in Met was significant ($p<0.04$). HbA1c decreased significantly in IG and Met (from 6.7 ± 1.1 to $6.3 \pm 1.1\%$, $p<0.04$ and from 6.1 ± 0.8 to $5.8 \pm 0.5\%$, $p<0.05$). In the IG group, this was associated with a trend to improved pp glucose (from 207 ± 96 to 161 ± 43 mg/dl, $p<0.052$) and reduced pp increase of glucose (from 72 ± 69 to 49 ± 40 mg/dl, $p<0.04$) and in Met with a trend to improved fasting glucose (from 124 ± 28 to 117 ± 15 mg/dl, $p<0.09$). Lipid profile and hsCRP were unchanged in both groups.

Conclusion: In patients with coronary artery disease and impaired glucose tolerance or early T2D, treatment with insulin glargine or metformin improved diastolic cardiac function, blood pressure and HbA1c. These results support efforts to start improving glucose metabolism in the early stage of pre-diabetes and diabetes. Given the prognostic importance of diastolic function and the relative improvement of intima media thickness with insulin glargine but not metformin, these results deserve further evaluation of cardiovascular endpoints and clarification of the underlying pathophysiological mechanisms.

Clinical Trial Registration Number: NCT01035528

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1058

Safety and efficacy of NPH after switching from insulin glargine in Canadian patients

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Background and aims: Due to provincial reimbursement restrictions, ACCORD patients treated with insulin glargine (IG) or insulin detemir (ID) will be required to switch to NPH. Switching takes place outside of the close monitoring of the trial. It was therefore decided to conduct a study to evaluate patient safety when IG is replaced with NPH.

Materials and methods: Patients leaving the ACCORD study treated with the long acting analogue insulins were randomized 1:1 to either IG or NPH insulin. Patients randomized to NPH were to use 80% of the prior IG dose, 80% of the prior ID dose administered qd, and 50% of the prior ID dose administered bid. It was investigator to administer NPH qd or bid. Follow

up as per clinical practice. Deviations would be captured as additional health resource utilization (HRU). The sample was predetermined by the number of available patients finishing ACCORD and not determined by the selection of primary outcome variable. As consequence, results are being presented using descriptive statistics. The primary outcome variable was the rate of confirmed symptomatic hypoglycemic events. Secondary outcomes included, rate of severe and nocturnal hypoglycemia, change from baseline in hemoglobin A1c (A1c), fasting blood glucose (FBG), weight and change from baseline in treatment satisfaction as measured by the status and change Diabetes Treatment Satisfaction Questionnaires (DTSQ). Poisson regression was used to analyze the semi-annual rate of symptomatic hypoglycemia and analysis of covariance was used to analyze the changes from baseline for the secondary variables. Analysis of covariance with treatment as a fixed effect and baseline value for the variable being analyzed was used to analyze change from baseline for the secondary variables.

Results: 32 patients randomized to IG and 34 to NPH both arms were comparable with respect to weight (median 98.25 kg), A1c (median 7.9%), FBG (7.9 mmol/L) and use of meal time insulin (59%). Median dose for prior IG was 50 units (range 8 to 140) and comparable for both treatment groups. Median dose for prior ID was 58 units (range 7 to 150). The 4 patients previously treated with ID were all randomized to the IG arm. Median doses of IG at 3 and 6 months were 42 and 47 units, respectively. Median doses of NPH at 3 and 6 months were 38 and 50 units, respectively. IG and NPH were administered bid in 4 and 2 patients, respectively. In all but one instance, the prior insulin had been administered bid. The semiannual rates (\pm se) per 100 patients for symptomatic hypoglycemia were 37.5 (2.2) IG and 31.1 (2.1) NPH. On the other hand, the semiannual rates for severe hypoglycemia per 100 patients were 2.7 (0.6) IG and 6.2 (0.9) NPH. There was no difference between the two groups for nocturnal hypoglycemia. The mean (\pm se) A1c decreases from baseline were -0.34% (0.11) IG and -0.01% (0.10), but no difference between treatments for FBG and weight. There was greater treatment satisfaction for the IG arm as measured by the change from baseline in the DTSQs and DTSQc. There was no difference observed for HRU between the two arms.

Conclusion: In this study of 66 randomized patients who had recently exited ACCORD, switching to NPH after having been previously treated with IG and ID, resulted in more than doubling the rate of severe hypoglycemia and less metabolic control. This, however, did not result in measurable difference in HRU.

Supported by: sanofi-aventis Canada

1059

Development of a novel insulin glargine chemiluminescent assay

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Background and aims: The long acting insulin analogue, insulin glargine, differs from human insulin by the addition of two arginine residues to the C-terminus of the B chain and the substitution of asparagine with glycine at the C-terminal end of the A chain. In most insulin assays, the consequence of these small changes is that glargine is either not measured or is measured with variable cross-reactivity. This makes it difficult to interpret the results when using insulin assays. Our aim was to develop a specific immunochemiluminometric (ICMA) assay using a solid phase capture antibody and a second, acridinium ester labelled, antibody for analysis of insulin glargine.

Materials and methods: We have developed a new monoclonal antibody to insulin glargine (RR19.5). The antibody was raised to a 13 amino acid peptide sequence, representing the C-terminus of the B-chain of glargine and coupled to bovine thyroglobulin carrier protein. RR19.5 was employed as either the solid phase capture antibody or as the labelled antibody, with 3B1, an antibody to insulin, as the corresponding antibody. Insulin glargine standards in the range of 0 - 250mU/L were prepared by diluting medicinal insulin glargine. Optimum assay format and incubation conditions, as demonstrated by the greatest relative light units (RLU) at the highest dose, with low background RLUs were determined. Cross-reactivity with human insulin and other insulin analogues was also determined.

Results: All assay formats produced dose responses to increasing insulin glargine standards. The optimum incubation conditions were found to be a single, 24 hour incubation at 4°C, employing the glargine specific RR19.5 as the solid phase antibody and the insulin antibody 3B1 as the label. Which-

ever assay format was used, cross reactivity was <3.5% for actrapid (human insulin), <3% for insulin glulisine <1.5% for insulin aspart, <1.5% for insulin detemir and <0.25% for insulin lispro.

Conclusion: We have developed a simple assay, specific for insulin glargine, that may prove useful in clinical applications and pharmaceutical development.

Supported by: WAG

1060

Novel indices for quantitative assessment of flatness of the time action profile of a basal insulin

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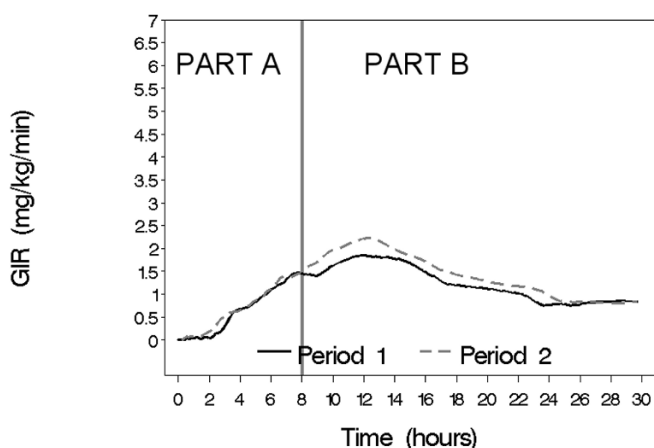
Background: Several quantitative parameters are available to define the time-action profile of a basal insulin. However, flatness a key feature of the TAP, is still largely determined qualitatively by visual inspection of the shape of the graphical representation of the time course profiles of glucose infusion rates (GIR) during an isoglycemic clamp. We propose 2 indices based on GIR excursion in an IGC, that could provide quantitative measures of flatness, 1) Mean Amplitude of GIR Excursion (MAGIRE), where excursions (absolute change from peak to nadir and vice versa) of GIR > 1 SD above mean GIR are presented as an average over the duration of action, and 2) Continuous Overlapping Net GIR (CONGIR), where for each observation after the first n hours of observations, the difference between the current GIR measurement and that in preceding hours is calculated. CONGIRn = SD of the differences and n = lag time (hours).

Materials and methods: Data from an open label test-retest time action profile assessment of a single subcutaneous dose of 0.4 units/kg of insulin glargine in a 30-hour isoglycemic clamp in 16 lean healthy subjects were analyzed, using the qualitative and indices-based approaches, and compared.

Results: Wherever the shape of graphical representation (Figure 1) of the time action profile was flat (Part B: 8–24 hrs), the calculated indices for both MAGIRE and CONGIRn were statistically significantly less than in periods where the shape was not flat (rapid change towards a peak) (Part A: 0–8 hrs). Thus MAGIRE was 0.75 in Part B vs 1.93 in Part A ($P < 0.001$), and CONGIR_{0.5} was 0.08 in Part B vs 0.13 in Part A ($p < 0.01$), indicating quantitatively more flat profiles in Part B vs Part A (see Figure 1).

Conclusion: These data suggest that these novel indices may provide a quantitative means for assessment of the flatness of the time action profile of a basal insulin. Further refinement of such indices could enable more accurate comparisons between basal insulins. Figure 1. Graphical Representation of the Time Action Profile of a Basal Insulin.

Median GIR during Clamp — with Loess Smooth



Clinical Trial Registration Number: NCT01152242

PS 094 Nephropathy: experimental

1061

Role of 12-lipoxygenase in slit diaphragm protein nephrin and P-cadherin expression in type 2 diabetic glomeruli

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Background and aims: 12-lipoxygenase (12-LO) was implicated in the development of diabetic nephropathy, in which the proteinuria was thought to be associated with a decreased expression of glomerular slit diaphragm protein nephrin and P-cadherin. Therefore, we investigated the role of 12-LO in the glomerular nephrin and P-cadherin expression in type 2 diabetic rats according to the glomerular sizes.

Materials and methods: 1) Rats fed with high-fat diet for 6 weeks were treated with low-dose streptozotocin. Once diabetes onset, diabetic rats were treated with 12-LO inhibitor cinnamyl-3,4-dihydroxy-cyanocinnamate (CDC) for 8 weeks. 2) Rats were randomly assigned to receive either 12(S)-HETE infusion or vehicle for 7 days by osmotic minipumps. 3) Rats were randomly assigned to receive either angiotensinII (AngII) infusion or vehicle by osmotic minipumps for 14 days. Glomeruli were isolated with a sieving method, using sieves with pore sizes of 250, 150, 125, and 75µm. We classified glomeruli into small (on the 75µm sieve) and large glomeruli (on the 125µm sieve) groups. Then, glomeruli were frozen in liquid nitrogen and stored at -70°C. RT-PCR, Western blotting, and immunofluorescent staining were used for mRNA and protein expressions of slit diaphragm protein and AngII type 1 receptor (AT1).

Results: We found that CDC did not affect the glucose levels but completely attenuated diabetic increases in glomerular volume and proteinuria. Diabetes significantly decreased the P-cadherin mRNA and protein expressions and increased the AT1 mRNA and protein expressions in the glomeruli compared to the control ($p < 0.01$). These changes were significantly prevented by CDC and recaptured by direct infusion of 12-LO product [12(S)-HETE] to normal rats for 7 days. The decreased P-cadherin expression was similar between large and small glomeruli, but the increased AT1 expression was significantly higher in the large than in the small glomeruli from diabetic and 12(S)-HETE-treated rats ($p < 0.01$). Nephrin mRNA and protein expressions were significantly reduced in the diabetic large glomeruli compared to the diabetic small glomeruli and control glomeruli ($p < 0.05$). In contrast, nephrin expression was significantly higher in the diabetic small glomeruli compared to diabetic large glomeruli and control glomeruli ($p < 0.05$). These changes were significantly prevented by CDC treatment. Direct infusion of normal rats with AngII for 14 days significantly decreased the glomerular P-cadherin expression compared to the control ($p < 0.01$). Direct infusion of normal rats with AngII and 12(S)-HETE significantly decreased large glomerular nephrin expression compared to the control ($p < 0.05$). However, small glomerular nephrin expression was increased after AngII and 12(S)-HETE treatment compared to the control ($p < 0.05$).

Conclusion: These results suggest that nephrin expression, but not P-cadherin expression, varies among the different sizes of glomeruli under type 2 diabetic conditions. Diabetic proteinuria is mediated by the activation of 12-LO-AT1 pathway that is partially attributed to the decreased glomerular nephrin and P-cadherin expression.

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1062

Prediabetic nephropathy caused by high-fat diet: relation to oxidative stress

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Background and aims: Both experimental and clinical studies suggest that diabetes-like complications develop at the prediabetic stage, prior to the development of overt hyperglycemia. The present study was aimed at characterizing renal changes in high-fat diet (HFD) fed mice, a model of prediabetes and alimentary obesity. We also evaluated the link between prediabetic kidney disease and oxidative stress, known to play a pivotal role in functional

and morphological manifestations of nephropathies, associated with both Type 1 and Type 2 diabetes.

Materials and methods: Male C57Bl6/J mice were fed normal mouse chow or high-fat (58 kcal% fat) diet for 16 wks. Urinary albumin and 8-isoprostane were evaluated by ELISA. Renal cortex TGF- β , VEGF, 4-hydroxynonenal adduct, and 12(S)hydroxyeicosatetraenoic acid [12(S)HETE] concentrations were measured by ELISA; CHOP, phospho-eIF2 α , and total eIF2 α (markers of endoplasmic reticulum stress), as well as nephrin, and 12/15-lipoxygenase expression by Western blot analyses; glucose and sorbitol pathway intermediates by enzymatic spectrofluorometric assays; NAD(P)H oxidase activity by chemiluminometry; PAS-positive substance and collagen deposition by histochemistry; and podocyte counts by immunohistochemistry.

Results: HFD feeding resulted in 46% increase in body weight, impaired glucose tolerance, and hyperinsulinemia. Kidney weight was increased by 11%. HFD-fed mice displayed polyuria, 2.7-fold increase in 24-h urinary albumin excretion, 20% increase in renal glomerular volume, 18% increase in renal collagen deposition, and 8% drop of glomerular podocytes. They also displayed a dramatic (5.3-fold) increase in urinary 8-isoprostane excretion and 38% increase in renal cortex 4-hydroxynonenal adduct accumulation, indicative of enhanced systemic and local oxidative stress. Studies of potential mechanisms of oxidative injury induced by HFD feeding revealed that whereas NAD(P)H oxidase activity only tended to increase, 12/15-lipoxygenase was significantly upregulated, with ~12% increase in the enzyme protein expression and ~2-fold accumulation of 12(S)HETE, a marker of 12/15-lipoxygenase activity. PAS-positive substance accumulation, TGF- β concentrations, and glucose and sorbitol pathway intermediate concentrations, as well as CHOP, phospho-eIF2 α , and total eIF2 α expression were indistinguishable between normal and HFD-fed mice. Surprisingly, renal cortex VEGF concentrations were reduced in HFD-fed mice, compared with controls.

Conclusion: HFD feeding causes prediabetic kidney disease, potentially via oxidative stress resulting from 12/15-lipoxygenase overexpression and activation.

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The crosstalk between monocyte and renal mesangial cells via interaction of metalloproteinases (MMP₂) and chemokines (Fractalkine)

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Background and aims: Previous work shown that advanced glycosylation end products (AGEs) increased the expression of fractalkine (Fkn) in human renal mesangial cells (HRMCs), and decreased the expression of MMP₂, furthermore, increased the chemotaxis and adhesiveness to the human monocytes (U937). The aim of this study was to further investigate the effect of Fkn on HRMCs treated with MMP₂, and effect of MMP₂ on U937 treated with Fkn.

Materials and methods: HRMCs was incubated with MMP₂ for 24h. U937 was incubated with recombinant human Fkn for 24h. The chemotaxis and adhesiveness of HRMCs to U937 was detected with a transwell system, co-culture and fluorescent staining respectively. The expression of Fkn was analyzed by RT-PCR and western blot, and the content in the supernatant of HRMCs was analyzed by ELISA. The expression of MMP₂ was analyzed by RT-PCR and gelatin zymograph.

Results: 1. Number of U937 transmigration to HRMCs treated with MMP₂ was increased dose dependently compared to control, but the adhesion number was decreased dose dependently (Table 1). 2. The expression of Fkn in HRMCs treated with MMP₂ (0.5, 1, 2, 3, 4, 5 ng/ml) was increased dose dependently, and the content in supernatant was decreased dose dependently (Table 1). 3. The expression of MMP₂ in U937 treated with Fkn (0.3 mg/L, 0.6 mg/L) was decreased compared to control (mRNA 0.48 ± 0.08 , 0.43 ± 0.09 vs 0.68 ± 0.21 ; Protein 1.15 ± 0.18 , 1.01 ± 0.13 vs 1.37 ± 0.15 ; $p < 0.05$). The decreased expression of MMP₂ in U937 treated with supernatant of HRMCs could be inhibited by Fkn-Ab ($p < 0.05$).

Conclusion: Fkn might up-regulate MMP₂ in U937 cells through HRMCs. Meanwhile MMP₂ increased the expression and chemotaxis of Fkn in HRMCs, but decreased the adhesiveness to the human monocytes. AGEs might participate in the interaction of Fkn and MMP₂, then contribute to the development of diabetic nephropathy.

Table 1 function and expression of Fkn in HRMCs treated with MMP₂ ($\bar{x} \pm s$)

MMP2(ng/mL)	control	0.50	1.00	2.00	5.00
chemotaxis	6.55 ± 0.51	14.21 ± 0.95	23.54 ± 1.56	34.48 ± 1.47	48.52 ± 3.55
adhesiveness	4.67 ± 0.88	3.33 ± 0.33	2.67 ± 0.56	2.00 ± 0.34	1.33 ± 0.45
Fkn mRNA	0.11 ± 0.09	0.80 ± 0.10	0.91 ± 0.09	1.04 ± 0.16	1.57 ± 0.33
Fkn Protein(ng/mL)	0.22 ± 0.03	0.27 ± 0.03	0.35 ± 0.05	0.63 ± 0.04	0.68 ± 0.01
Fkn ELISA(ng/mL)	116.60 ± 3.00	100.64 ± 1.78	75.32 ± 0.97	68.17 ± 1.31	52.28 ± 1.34

Compared to the control group, all the data has significant difference ($p < 0.05$)

1064

Hyperglycaemic E1-DN mice develop albuminuria and glomerular injury

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Background and aims: The transgenic E1-DN mice express a kinase-negative epidermal growth factor receptor (EGF-R) in their pancreatic islets and are diabetic due to impaired postnatal growth of pancreatic islet β -cells. The mice are viable and survive without insulin treatment, and can be used to investigate the effects of long term exposure to hyperglycaemic environment. The aim of this study was to characterize the development of renal injury in these diabetic mice.

Materials and methods: The transgenic mice in FVB background were generated previously. E1-DN homozygous (n=13, n=9), heterozygous (n=14, n=14) and wildtype (n=17, n=12) mice were followed to the age of 20 or 40 wks. Blood glucose was measured with glucometer, and 24 hour urine samples were collected in metabolic cages, urine volume was measured and albumin concentration analyzed by ELISA. Apoptosis was detected with caspase 3 immunoperoxidase and podocyte proteins with immunofluorescence stainings.

Results: An increase in albumin excretion rate (AER) in homozygous E1-DN mice was detected at the age of 10 wks when compared to wt mice. (AER 330 μ g/24h vs. 135 μ g/24h, $p < 0.01$) At 20 wks the increase continued. (AER 1257 μ g/24h vs. 282 μ g/24h, $p < 0.01$) At 40 wks some of the hyperglycaemic mice developed massive proteinuria, the highest AER being 64mg/24h. Histological stainings (HE, PAS) showed glomerulosclerosis in the hyperglycaemic mice. Apoptosis of glomerular cells was detected more often in homozygous E1-DN mice when compared to heterozygous and wt mice. (6.5 vs. 1 apoptotic cells / 10 glomeruli at 20 wks, and 9.8 vs. 4.4 apoptotic cells / 10 glomeruli at 40 wks, $p < 0.05$) The expression patterns of several podocyte proteins (nephrin, CD2AP, podocin and ZO-1) were not altered.

Conclusion: Hyperglycaemic E1-DN mice develop albuminuria and glomerular injury resembling human diabetic nephropathy and can serve as a new model to study diabetic nephropathy. Increased apoptosis is identified as one mechanism contributing to glomerular injury.

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High glucose differentially modulates the inhibitory effects of metformin and rapamycin on mTOR signalling

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Background and aims: There is evidence that the activation of the mTOR pathway plays an important role in diabetic nephropathy. According to clinical studies in both type 1 and type 2 diabetes, longstanding hyperglycaemia is the primary cause of diabetic nephropathy. However, the molecular mechanism of high glucose-induced mTOR activation is poorly understood. This study investigated the effects of mTOR inhibitors, metformin and rapamycin, on proliferation, cell size, and mTOR pathway activation induced by excess D-glucose and L-leucine.

Materials and methods: HEK293 cells were exposed to D-glucose (30 mM) and L-leucine (10 mM), in the presence and absence of metformin (8 mM) and rapamycin (100 nM). Cell size and proliferation were measured by flow cytometry. The activation status of the mTOR signalling pathway was determined by immunoblotting. Viability was determined by standard metabolic assays and microscopy. Data were obtained from at least three independent experiments. Statistical significance was determined at $P < 0.05$; ANOVA.

Results: L-leucine and D-glucose increased cell size (100 ± 2.8 to 114 ± 2.8 %, $P<.01$ and 100 ± 1.5 to 122.2 ± 6.7 %, $P<.05$ respectively). Both metformin and rapamycin decreased cell size in L-leucine- (114 ± 2.8 to 64.7 ± 2.9 and 59.5 ± 3.2 %, $P<.001$) and D-glucose-treated cells (122.2 ± 6.7 to 102.5 ± 5.8 and 97.7 ± 4.4 %, $P<.05$). Relative to controls metformin and rapamycin increased the G0/G1 phase of the cell cycle (55 ± 0.8 to 66.2 ± 0.5 and 61.7 ± 0.5 % respectively, $P<.01$). D-glucose pre-treatment abrogated the inhibitory effect of metformin on cell cycling but had no effect on cell cycle inhibition by rapamycin (57.7 ± 0.5 , $P>0.05$ and 62 ± 1.6 , $P<.01$ respectively). These differential effects of metformin and rapamycin on cell cycling were reflected in the activation level of mTOR assessed by immunoblotting.

Conclusions: Our results suggest that high glucose-mediated cell size increase is sensitive to inhibition by both metformin and rapamycin. However, high glucose differentially affected metformin- and rapamycin-induced cell cycle arrest and mTOR inhibition. Our findings may lead to novel insights into the mechanisms of glucose-mediated cellular hypertrophy and the role of mTOR signalling in diabetic nephropathy.

Supported by: PCMD

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R3h-domain containing like protein is a novel regulator of glomerular basement membrane

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Background and aims: Recent discoveries of podocyte expressed genes have dramatically increased our knowledge of the molecular mechanism of glomerular filter of the kidney. In order to increase our knowledge about glomerular functions, we have previously identified >300 glomerulus-enriched transcripts through large-scale sequencing and microarray profiling of the mouse glomerular transcriptome. One of the podocyte-specific transcripts identified was R3h-domain containing like (R3hdm1). The aim of this study is to analyze the functions of R3hdm1 both *in vivo* and *in vitro*.

Materials and methods: The expression patterns of R3hdm1 mRNA as well as protein were examined by RT-PCR and by the immunohistochemistry using anti R3hdm1 specific antibody. The R3hdm1 knockout mice were created in order to know the function of R3hdm1 *in vivo*.

Results: R3hdm1 was exclusively podocyte's specific, since we could not detect R3hdm1 transcripts other than the glomeruli analyzed by RT-PCR and its expression were increased in diabetic condition. The R3hdm1 gene encodes 253 amino acids composed of a SCP-like extracellular domain which has been proposed to be a Ca chelating serine protease. However function of this protein in mammals is unknown. Anti Rh3dml specific antibody localized R3hdm1 protein to peri-nuclear and/or in the nucleus. Light microscopic analysis revealed that the R3hdm1 null mice had no observable glomerular developmental defects. However, an electron microscopic study revealed thickening of the glomerular basement membrane (GBM) and effacement of the podocyte foot processes which mimicked the diabetic glomerulosclerosis. When they are induced diabetes by the injection of streptozotocin, R3hdm1 null mice produced more albuminuria and decreased renal functions than the wild type controls.

Conclusion: We identified R3hdm1 as a novel podocyte's specific gene which might have a role in the development of diabetic glomerular disease.

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Dietary restriction-induced Sirt1 activation improves diabetic nephropathy through anti-inflammatory action and autophagy in diabetic wistar fatty rats

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Background and aims: Dietary restriction (DR) or the activation of Sirt1 induced by DR exerts anti-inflammatory effect and regulates autophagy, lead-

ing to the life expansion. The micro-inflammation has been implicated in the initiation and progression of diabetic nephropathy, and it is also shown that the regulation of autophagy associated with Sirt1 is impaired in the aging kidney. However the effects of DR on nephropathy of type 2 diabetes remains elusive. The aim of this study is to investigate whether DR exerts the reno-protective effects in Wistar fatty rats, a model of type 2 diabetes.

Materials and methods: Wistar fatty rats were treated with DR (40% restriction) for 24 weeks. We measured urinary albumin excretion (UAE), creatinine clearance (Ccr), histological changes including Periodic acid-Schiff staining (PAS) and Masson-Trichrome staining and immunohistochemistry for ED-1, a marker of macrophage. mRNA expression of MCP-1, VCAM-1, ICAM-1, TGF- β 1, fibronectin and collagen IV by real-time PCR, and acetylated-NF- κ B (p65) and Sirt1 protein expression by western blotting were also estimated in the renal cortex. In addition, autophagy was assessed by western blotting of p62/sequestosome 1 (SQSTM1), a marker for *in vivo* damaged autophagy, and electron microscopic observation of mitochondrial morphological change. Blood glucose, glycated-Hb and plasma lipid profiles were also measured.

Results: DR significantly reduced UAE and attenuated renal histological changes in Wistar fatty rats. The mRNA expression of inflammation- and fibrosis-related genes were almost completely improved to the control levels of non diabetic Wistar lean rats. The increase of acetylated-NF- κ B and p62/SQSTM1 were observed in Wistar fatty rats. We also revealed that the Sirt1 expression was decreased and mitochondria morphology was altered, resulted in swelling and disintegration of cristae in the renal cortex of Wistar fatty rats. DR ameliorated the alterations of NF- κ B, p62/SQSTM1, Sirt1 expression, and mitochondrial changes seen in the kidney of Wistar fatty rats. The increased blood glucose, glycated-Hb, and abnormal lipid profiles in Wistar fatty rats were partially improved by the treatment with DR.

Conclusion: DR could exert anti-inflammatory effects and improve the regulation of autophagy through restored Sirt1 activation in the kidney of Wistar fatty rats, resulting in the amelioration of renal injuries in type 2 diabetic kidney.

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Dietary restriction retards ageing and diabetes-related kidney injury

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Background and aims: Aging is a biological process and is associated with various metabolic disorders, including type 2 diabetes. The incidence of type 2 diabetes has risen considerably in the past 2 decades and diabetic nephropathy is one of the most important microvascular complications observed in diabetes. It is known that dietary restriction has an important role in preventing age-related diseases. The main purpose of the study was to analyze the effect of dietary restriction on kidney function related with aging and type 2 diabetes.

Materials and methods: Male control Wistar and diabetic Goto-Kakizaki rats, with 12 months of age, were divided in four groups: two groups of Wistar (W) and GK rats (GK) fed ad libitum and two groups of Wistar (WDR) and GK rats (GKDR) with dietary restriction up to 35% during 4 months. We analyzed the metabolic profile, pro-inflammatory [tumor necrosis factor- α (TNF- α), interleukin (IL)-6, interleukin (IL)-1 β] and fibrotic [transforming growth factor (TGF)- β 1] markers, AMP-activated protein kinase (AMPK) and sirtuin-1 (SIRT1) in all groups by ELISA and Western Blot techniques.

Results: Dietary restriction significantly reduced (22%, $p<0.001$) body weight in GK rats, and glycaemia 2h after glucose load (20% and 27%, $p<0.01$) and HbA1c (6%, $p<0.05$; 16%, $p<0.001$) in normal and diabetic rats. Dietary restriction significantly improved triglycerides (27%, $p<0.05$; 60%, $p<0.001$) and free fatty acids (FFAs) (71%, $p<0.001$; 45%, $p<0.05$) in normal and diabetic rats. Furthermore, dietary restriction reduced the levels of all pro-inflammatory and pro-fibrotic markers in Wistar (TNF- α 25%, $p<0.05$; IL-1 β 25%, $p<0.01$; IL-6 31%, $p<0.01$; TGF- β 1 5%, $p<0.05$) and GK rats (TNF- α 50%, $p<0.01$; IL-1 β 33%, $p<0.01$; IL-6 32.5, $p<0.05$ and TGF- β 1 5%, $p<0.05$). Noteworthy, the ratio phospho-AMPK/AMPK (38% and 33%) and the levels of SIRT1 (24% and 21%) in normal and diabetic rats were significantly increased by dietary restriction ($p<0.05$).

Conclusion: The present study suggests that dietary restriction improves the metabolic profile and kidney function by a mechanism that apparently involves SIRT1 and AMPK in aged normal W and diabetic type 2 GK rats.

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K-21299, a novel angiotensin II receptor blocker and selective PPAR γ agonist, is more effective than telmisartan in improving diabetic nephropathy in ZDF rats

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Background and aims: Angiotensin II (AII) receptor blockers (ARBs) have been shown to be of benefit for the treatment of diabetic nephropathy. Recently, peroxisome proliferator-activated receptor- γ (PPAR γ) agonists have also been demonstrated to slow the progression of diabetic nephropathy. Telmisartan is a unique ARB with a PPAR γ agonistic action, which may contribute to the beneficial pleiotropic effects. We have developed a novel ARB, K-21299, which has strong PPAR γ agonistic action compared with telmisartan. This study was performed to evaluate the efficacy of K-21299 for use in the treatment of diabetic nephropathy.

Materials and methods: Zucker diabetic fatty (ZDF) rats were orally administered K-21299 (1, 3, and 10 mg/kg/day), telmisartan (3 mg/kg/day) or vehicle alone for 15 weeks. Urine and blood samples were collected for measurement of urinary total protein (UTP) excretion and for biochemical analysis, respectively. BP was measured with the tail cuff method. Fifteen weeks after administration, the kidneys were excised for histological assessment and liver samples were collected for gene expression analysis.

Results: Progressive proteinuria was significantly suppressed by K-21299 in a dose-dependent manner, whereas the reduction of BP by K-21299 was similar regardless of the dose. K-21299 induced a more marked decrease in UTP than the same dose of telmisartan. Moreover, the histological glomerular damage was also ameliorated by K-21299. The results of biochemical analyses indicated that K-21299 decreased plasma triglyceride, total cholesterol, non-esterified fatty acid, LDL-cholesterol and uric acid levels, but not plasma glucose levels. In addition, sterol regulatory element binding protein 1c mRNA and the expression levels of genes associated with fatty acid synthesis were down-regulated by K-21299 in the liver. On the other hand, telmisartan decreased only plasma total cholesterol levels, but did not affect the other blood biochemical parameters.

Conclusion: K-21299 markedly ameliorated progressive diabetic nephropathy in ZDF rats. Furthermore, the renal protective effect of K-21299 was independent of the antihypertensive effect, possibly due to its potentiated PPAR γ agonistic action compared with telmisartan. These results suggest that K-21299 may be more beneficial than other ARBs for use in the treatment of diabetic nephropathy.

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Nitric oxide bioavailability is ameliorated by green tea in experimental diabetes mellitus

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Background and aims: In diabetes mellitus (DM) nitric oxide (NO) deficiency contributes to diabetic nephropathy (DN). In these settings, NO deficiency is the result of reduced NO production by uncoupled endothelial nitric oxide synthase (eNOS), and increased consumption, secondary to ligation of NO with superoxide (O_2^-), forming peroxynitrite. Tetrahydrobiopterin (BH_4) levels are essential to maintain the eNOS coupled. Uncoupled eNOS produces O_2^- instead NO, contributing to oxidative stress. It has been demonstrated that in experimental DM green tea (GT; *Camellia sinensis*) ameliorates DN by reducing oxidative stress. However the effect of GT in NO bioavailability and synthesis in experimental DM is unknown. The aim of the present study was to investigate the potential of GT to ameliorate NO bioavailability in diabetic conditions.

Material and methods: Twelve-week-old spontaneously hypertensive rats (SHR) were rendered diabetic by intravenous injection of streptozotocin (50 mg/kg), whereas control rats received citrate buffer. Diabetic SHR rats were randomized to receive no treatment or treatment with daily, freshly prepared, GT (1.7g/kg body weight/day). After 12 weeks of treatment the levels of NO were determined by nitrate/nitrite. BH_4 was measured in urine and renal cortex samples using UPLC (Ultra Performance Liquid Chromatography). For *in vitro* studies, human mesangial cells (HMC) were cultured in normal glucose (NG; 5mM) and high glucose (HG; 30mM) with or without GT treatment (100ug/ml). Oxidative stress was assessed by DCF-DA and NADPH oxidase induced O_2^- generation by lucigenin.

Results: The systolic blood pressure did not differ between groups of the study. However, body weight was less ($p < 0.0001$) and glycemia was greater in diabetic SHR rats (treated or not with GT) than in nondiabetic rats ($p < 0.0001$). In diabetic animals, there was a reduction in NO bioavailability, which was reversed with GT. There was no difference in the renal eNOS expression between the groups, but the expression of p-Thr497eNOS, an indication of inactivation of eNOS, was increased in diabetic animals and decreased by GT treatment. Total biopterin, BH_4 and oxidation rate of BH_4 in urine and renal cortex were significantly reduced ($p < 0.0001$) in diabetic rats and reversed by GT ($p < 0.05$). In HMC, cultured under HG, there was a rise in reactive oxygen species (ROS) production ($p < 0.001$). The main source of ROS, in HMC under HG, was NADPH oxidase ($p < 0.0001$), but eNOS uncoupling also plays a key role in HG-induced oxidative stress ($p = 0.04$). Treatment of HMC with GT reversed the rise in ROS ($p < 0.001$). NO levels were reduced by HG and were restored with GT treatment. Both blockade of the biosynthesis of BH_4 and recycling of 7,8-dihydro-L-biopterin (BH_2) to BH_4 increased ROS production in both NG and HG conditions ($p < 0.0001$), and they were restored with GT treatment ($p < 0.001$). Addition of BH_4 to HMC exposed to HG also decreased ROS production ($p = 0.005$). However, treatment of HMC with GT plus BH_4 did not reduce ROS production compared to HMC cultured only with GT, suggesting that GT may act in synthesis or recycling of BH_4 .

Conclusion: In summary, green tea increased NO bioavailability by restoration of BH_4 production, coupling eNOS and reducing oxidative stress, abnormalities that are involved in the pathogenesis of diabetic nephropathy.

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PS 095 Nephropathy: risk factors and biomarkers

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Advanced glycation end products increase human mesangial foam cell formation by SCAP dysfunction

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Background and aims: Diabetic nephropathy caused by advanced glycation end products (AGEs) is associated with lipid accumulation in glomeruli. This study was designed to investigate whether N^ε-(carboxymethyl) lysine (CML, a member of the AGEs family) increases lipid accumulation in human mesangial cells (HMCs) via the LDL receptor pathway by increasing SREBP cleavage-activating protein (SCAP) transcription and its glycosylation in the Golgi apparatus.

Materials and methods: Intracellular cholesterol content was assessed by Oil Red O staining and cholesterol assay in HMCs treated with CML. mRNA and protein levels of molecules controlling cholesterol homeostasis were examined in the treated cells using real-time quantitative RT-PCR and western blotting, respectively. The activity of Golgi processing enzymes was determined using enzyme-based methods and the translocation of SCAP from the endoplasmic reticulum (ER) to the Golgi was detected by confocal microscopy.

Results: CML increased cholesterol accumulation in HMCs. Exposure to CML increased expression and abnormal translocation of SCAP from ER to Golgi even in the presence of a high concentration of LDL. The increased SCAP translocation carried more transcription factor SREBP-2 to the Golgi for activation by proteolytic cleavage, enhancing transcription of HMGCoA reductase and the LDL receptor. Furthermore, CML enhanced SCAP glycosylation by upregulating Golgi mannosidase activity. This prolonged the half-life and enhanced recycling of SCAP between the ER and the Golgi. The effects of CML were blocked by inhibitors of Golgi mannosidases.

Conclusion: AGEs (CML) increased lipid synthesis and uptake, thereby causing foam cell formation via increasing transcription and protein glycosylation of SCAP by Golgi enzymes in HMCs. These data imply that inhibitors of Golgi processing enzymes might have a potential renal protective role in prevention of mesangial foam cell formation.

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Higher serum high-mobility group box 1 levels are cross-sectionally associated with micro- and macroalbuminuria, but not with cardiovascular disease in type 1 diabetes: the EURODIAB PCS

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Background and aims: High-mobility group box 1 (HMGB1) is a pro-inflammatory cytokine that may contribute to the pathogenesis of vascular complications commonly observed in diabetes. In another study we found that plasma HMGB1 was associated with incident cardiovascular disease (CVD) in patients with type 1 diabetes. Whether HMGB1 is also associated with microvascular complications is unclear. Therefore, we investigated whether serum HMGB1 is associated with: (1) the presence and severity of nephropathy and retinopathy, and prevalent CVD in type 1 diabetes; and (2) the potential mediating roles of markers of low-grade inflammation (LGI) and endothelial dysfunction (ED), and pulse pressure (PP, a marker of arterial stiffness) therein.

Materials and methods: We included 463 patients (226 women; mean age 40±10 yrs) with type 1 diabetes from the EURODIAB Prospective Complica-

tions Study. We used linear and binary or multinomial logistic regression analyses adjusted for age, sex, HbA_{1c}, duration of diabetes, body mass index, total cholesterol, triacylglycerols, mean arterial pressure, antihypertensive treatment, smoking, and presence of CVD, albuminuria and/or retinopathy as appropriate.

Results: Serum Ln-HMGB1 levels were positively associated with LGI and ED [standardised regression coefficient $\beta=0.07$ (95% CI: 0.02 to 0.12) and $\beta=0.08$ (0.02 to 0.15), respectively], but not with PP. Higher serum Ln-HMGB1 levels (per unit increase) were associated with greater odds of micro- and macroalbuminuria: OR=1.23 (0.90 to 1.69) and OR=1.63 (1.18 to 2.24), respectively. Further adjustments for markers of LGI or ED did not attenuate these associations. Serum Ln-HMGB1 levels were not associated with estimated glomerular filtration rate by Cockcroft-Gault formula [eGFR, $\beta=-0.04$ (-0.11 to 0.03)], background and proliferative retinopathy [OR=0.93 (0.69 to 1.25) and OR=0.89 (0.64 to 1.25), respectively] or CVD [OR=0.87 (0.67 to 1.12)], however.

Conclusion: This study shows that in individuals with type 1 diabetes higher serum HMGB1 levels are associated with greater prevalence and severity of albuminuria, though not with eGFR, retinopathy, and CVD. However, this finding of no association between serum HMGB1 levels and prevalent CVD together with the positive association between plasma HMGB1 and incident CVD observed in our other study may suggest that serum levels and plasma levels of HMGB1 do not represent the same pool of HMGB1. Further studies are needed to clarify both the interrelationship between serum and plasma levels of HMGB1, and their roles, if any, in the pathogenesis of vascular complications in type 1 diabetes.

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Diabetic nephropathy in T1DM is associated with an altered expression of genes regulating TGF-beta signalling and fibrosis, apoptosis and cell cycle. Studies in primary cultures of human fibroblasts

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Background and aims: Diabetic nephropathy (DN) has a genetic component, still largely unknown. The search for genetic markers in predisposed individuals can help in the identification of individuals at risk, as well as in the understanding of the underlying patho-physiological processes leading to DN. To these purposes, we have determined the whole transcriptome in cultured human fibroblasts after 8 passages in culture, derived from T1DM patients with a long disease duration (>15 years), either with established DN (DN+, n = 5), or without DN (DN-, n = 5), as well as in healthy controls (n = 5), by a microarray technique.

Materials and methods: All the subjects were matched for age, sex, and BMI, and the two groups of T1DM subjects also for metabolic control (HbA_{1c} in DN+ : 10.5±1.3%; DN- : 9.3±0.8%), as well as for diabetes duration (DN+ : 21.8±4.5 yrs; DN- : 22.8±3.2 yrs). The comparison between groups was performed by ANOVA, with a level of significance set at p<0.01. Furthermore, over- and under-expressed genes were selected using a cut-off fold change of >3.

Results: With respect to control subjects, the largest differences as regards both the number of genes and the magnitude of the differences, were found in the cultured fibroblasts of the T1DM patients with DN. 197 genes were under-expressed, whereas 89 were over-expressed. The genes showing the largest differences between T1DM patients and controls were ranked according to the magnitude of change. Thereafter, they were grouped also on the basis of their function. The main alterations in expression were found in genes regulating TGF-beta signalling and fibrogenesis, (n = 4, among them thrombospondin-1), apoptosis (n = 13, among them interleukin7, coagulation factor II receptor) and cell cycle (n = 36, among them the cyclin-dependent kinases CDC45L and CDKN2C).

Conclusion: We report a number of differences in gene expression in cultured fibroblasts of T1DM subjects with vs. without DN, as well as with respect to control subjects. Since the microarray study was performed in cells kept in culture for many passages, it is unlikely that preceding, in vivo environmental factors accounted for the differences observed. Furthermore, the degree of metabolic control, based on HbA_{1c} value, was similar between two diabetic groups. Therefore, these findings can help both in the identification of individuals genetically predisposed to DN, as well as in the understanding of the patho-physiological mechanisms leading to DN.

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Incretin effect and gastrointestinal-mediated glucose disposal in patients with end-stage renal disease and normal or impaired glucose tolerance

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Background and aims: Type 2 diabetes mellitus (T2DM) is characterized by a severely impaired incretin effect. Patients with T2DM and non-diabetic patients with end-stage renal disease (ESRD) share several pathophysiological traits: insulin resistance, hyperinsulinaemia and impaired beta cell function. We aimed to determine the incretin effect in non-diabetic patients with ESRD treated with chronic maintenance haemodialysis.

Materials and methods: The incretin effect was measured in 10 patients with ESRD and normal glucose tolerance (NGT), 10 patients with ESRD and impaired glucose tolerance (IGT) and 11 healthy control subjects (CTRL), using 3-hour 75 g-oral glucose tolerance test (OGTT) and isoglycaemic intravenous glucose infusion (IIGI) on separate days. Gastrointestinal-mediated glucose disposal (GIGD) based on glucose amounts utilized ($100 \times (\text{glucose}_{\text{OGTT}} - \text{glucose}_{\text{IIGI}}) / \text{glucose}_{\text{OGTT}}$) and incretin effects based on area under curve (AUC) for insulin ($100 \times (\text{AUC}_{\text{OGTT}} - \text{AUC}_{\text{IIGI}}) / \text{AUC}_{\text{OGTT}}$) were calculated.

Results: All groups were matched by age, body mass index and gender. The amount of glucose needed to obtain isoglycaemia during IIGI was significantly ($p < 0.02$) greater in both of the uraemic groups (46 ± 17 g (NGT) and 52 ± 8 g (IGT), $p = 0.4$ between groups) compared to CTRLs (31 ± 6 g). Correspondingly, GIGD was found to be significantly ($p < 0.02$) diminished in the uraemic groups ($38 \pm 7\%$ (NGT) and $31 \pm 3\%$ (IGT), $p = 0.4$ between groups) compared to the CTRL group ($59 \pm 3\%$) (Fig. 1). Incretin effects amounted to $64 \pm 16\%$ (CTRL), $54 \pm 15\%$ (NGT) and $38 \pm 26\%$ (IGT), respectively, with significant difference between CTRLs and IGTs ($p = 0.01$).

Conclusion: ESRD patients with NGT or IGT are characterised by reduced GIGD compared to subjects with NGT and normal kidney function and the incretin effect is significantly impaired in ESRD patients with IGT.

GIGD

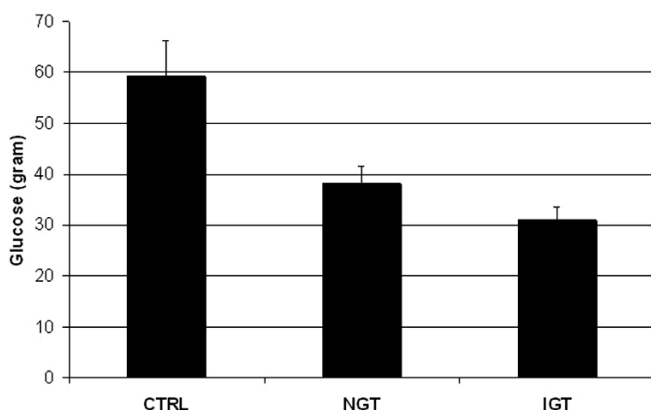


Fig. 1 GIGD values

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Evaluation of bone mineral density in long-standing type 1 diabetic patients with or without diabetic nephropathy

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Background and aims: Patients with chronic kidney disease including diabetic nephropathy have been suggested to have a lower bone mineral density (BMD). The aim of the study was to measure bone mineral density (BMD)

and evaluate the prevalence of osteoporosis in long-standing type 1 diabetic patients with or without overt diabetic nephropathy.

Materials and methods: Cross sectional evaluation of a prospectively followed cohort of 293 long-standing, type 1 diabetic patients. From 1/2-2011 to 31/3-2011, 115 patients with diabetic nephropathy (54% men; age [mean±SD] 54 ± 9 years, 42 ± 8 years of diabetes, duration of nephropathy 22 ± 6 years) and 178 patients with persistent normoalbuminuria (49% men, age 59 ± 10 years, 41 ± 10 years of diabetes) were included. BMD (g/cm^2) in femoral neck, total femoral bone and lumbar spine was measured by dual energy x-ray absorptiometry (DXA), (Hologic, Discovery A). Osteopenia and Osteoporosis were defined by any sex matched T-score ranging from -2.5 to -1.0 and < -2.5 , respectively. The difference between the measured BMD and age-sex matched average defines the Z-score. GFR was measured by ^{51}Cr -EDTA plasma clearance in patients with nephropathy.

Results: Among patients with diabetic nephropathy, 58 (50%) and 31 (27%) had T-scores corresponding to osteopenia and osteoporosis, respectively compared to 106 (60%) and 22 (12%) of the normoalbuminuric patients; $p = 0.006$. Among male patients with nephropathy, the prevalence of osteoporosis was 37% vs. 9% with normoalbuminuria ($p < 0.001$), whereas no difference was seen among women. Similarly, men with nephropathy had significantly lower age-sex matched spinal, femoral neck and total femoral bone Z-scores compared to normoalbuminuric patients; $p \leq 0.002$. Among women, there was no statistically difference. Finally, among patients with diabetic nephropathy, baseline GFR levels did not differ between men and women (94 ± 29 vs. 95 ± 30 ; $p = 0.92$), respectively but correlated positively with femoral neck and total femoral BMD ($r = 0.36$ and $r = 0.38$; $p < 0.001$, respectively). Collection of data regarding the prevalence of diagnosis/treatment of osteoporosis and history of fractures is ongoing.

Conclusion: The risk of osteoporosis was highest among male patients with type 1 diabetes and long-standing diabetic nephropathy and femoral BMD correlated with renal function. Hence screening, prevention and treatment of osteoporosis in patients with impaired renal function should be considered.

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Serum bilirubin levels and the changes in kidney function in type 2 diabetic patients

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Background and aims: Oxidative stress has been implicated in the pathogenesis of diabetic vascular complications, including diabetic kidney disease (DKD). Bilirubin is a potent antioxidant that effectively scavenges peroxyl radicals and suppresses the oxidation of lipids and lipoproteins. In patients with type 2 diabetes mellitus (T2DM), serum bilirubin levels were shown to be positively correlated with estimated glomerular filtration rate (eGFR). Another report documented decreased serum bilirubin levels to be associated with reduced eGFR only in women. However, these studies were limited by cross-sectional design. We therefore conducted this longitudinal study to clarify the association between serum bilirubin levels and the deterioration of kidney function in T2DM patients. Gender differences in the association were also determined.

Materials and methods: This was a single institutional observational cohort study, including Japanese adult T2DM patients with normoalbuminuria, defined as urinary albumin-to-creatinine ratio (ACR) < 30 mg/g. Subjects with malignant disease, liver cirrhosis, hematologic disease, and those with serum bilirubin ≥ 1.2 mg/dL were excluded. Patients were followed up for at least 2 years. The primary outcome measurement was the rate of change in eGFR. For statistical analyses, multiple regression analysis and analysis of covariance (ANCOVA) were conducted.

Results: Overall, 1,441 patients, 599 women and 842 men, with a mean (\pm SD) age of 59 ± 12 years, were studied. The mean follow-up period was 5.4 ± 1.1 years (range: 2.0–7.1) years and the mean annual rate of change in eGFR in the entire cohort was -0.76 ± 1.96 mL/min/1.73 m^2 /year. A significant interaction between serum bilirubin levels and gender on the primary outcome was detected (p interaction = 0.045); therefore, women and men were analyzed separately. In women, the univariate regression analysis showed a significant correlation between serum bilirubin levels and the rate of change in eGFR ($r_s = 0.080$, $p = 0.049$), indicating that higher serum bilirubin levels were associated with slower decline in eGFR. In the multivariate regression analysis adjusted for other clinical factors, serum bilirubin level remained as a statistically significant variable associated with the rate of change in eGFR (standardized parameter estimate = 0.100, $p = 0.013$). In men, there was no significant association between serum bilirubin levels and the rate of change

in eGFR in either univariate or multivariate analysis. Next, we classified subjects into gender-specific tertiles according to bilirubin levels to clarify the effect of bilirubin treating as a categorical variable. In women, the adjusted rate of change in eGFR in the third bilirubin tertile (-0.36 ± 0.14 mL/min/1.73 m²/year) was significantly slower than in the first tertile group (-0.84 ± 0.16 mL/min/1.73 m²/year, $p = 0.027$) and the second tertile (-0.79 ± 0.12 mL/min/1.73 m²/year, $p = 0.022$). In men, there were no significant differences in the adjusted rate of change in eGFR among the 3 tertiles.

Conclusion: Higher levels of serum bilirubin may be predictive of slower deterioration of kidney function in diabetic women but not in men.

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CKD categories by KDIGO and the progression rate in GFR, albuminuria and cardiovascular disease in type 2 diabetes without prevalent cardiovascular disease: prospective cohort study

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Background and aims: The continuum of development, progression and complications of diabetic renal disease is not fully understood. The aim of this study was to investigate the risk in terms of the stage progression rate in albuminuria and eGFR and the cardiovascular occurrence rate based on the categories by albuminuria and eGFR through a large nationwide prospective cohort study in Japan.

Materials and methods: A prospective study was performed in primary care settings at multiple clinics nationwide. Participants were 2,984 individuals with type 2 diabetes without prevalent cardiovascular disease. CKD was defined according to the KDIGO classification, and stage 3 CKD was further classified into stage 3N (with normoalbuminuria) and 3M (with micro/macrolbuminuria).

Results: During a median follow-up of 3.8 yr, the incidence rate of cardiovascular event was 8.3 (95% CI 6.7–9.9) per 1,000 person years. 1) Compared to No CKD as a reference group, individuals with stage 2 and stage 3M CKD had a significantly increased risk of adverse cardiovascular outcomes ($p < 0.05$, $p < 0.01$, respectively). 2) Individuals with stage 1 and stage 3N did not reveal any significant risk of adverse cardiovascular outcomes, while those with stage 1 had a high rate of progression to stage 2 (40% per 4-year). 3) Progression rate in albuminuria stage was similar between No CKD and Stage 3N (10% and 12% per 4-year, respectively). Progression rate in GFR stage was more than three-fold higher in subjects with albuminuria (Stage 2 and 3M, 17% and 10% per 4-year, respectively) than in those with normoalbuminuria (No CKD and 3N, 5% and 2% per 4-year, respectively). 4) Among subjects with No CKD, those of whom albuminuria stage progressed during follow-up (transited from normoalbuminuria to albuminuria) showed a significantly increased risk of adverse cardiovascular outcomes ($p < 0.01$), whereas those of whom GFR stage progressed did not.

Conclusion: This prospective cohort study of Japanese type 2 diabetes indicates that development of microalbuminuria plays a crucial role as an initial

step to the occurrence of renal function loss and cardiovascular disease, while further follow-up is ongoing.

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Cardiovascular risk factors and complications associated with impaired renal function and albuminuria in insulin-treated type 2 diabetes

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Background and aims: Albuminuria (AU) and reduced estimated GFR (ReGFR) predict cardiovascular (CV) events in type 2 diabetes. In this study, we investigated the association of AU and ReGFR with CV risk factor control, treatment and prevalent complications in longstanding insulin-treated type 2 diabetic patients.

Materials and methods: Cross-sectional study among 5100 insulin-treated (≥ 2 injections/day) type 2 diabetic patients visiting Belgian secondary care diabetes centres in 2009. AU was defined as ≥ 20 µg/min, ≥ 30 mg/24 h, ≥ 30 mg/g creatinine, or ≥ 20 mg/L. ReGFR was defined as eGFR < 60 mL/min/1.73 m².

Results: At least 80% of patients received antihypertensive drugs and 70% received lipid-lowering drugs (see table). Number of patients treated with antihypertensive drugs increased as AU, ReGFR or both were present. By contrast, lipid-lowering treatment was associated only with ReGFR, and not with AU. The higher rate of lipid-lowering treatment among ReGFR patients was associated with higher use of fibrates (9.3% vs. 6.5% in patients without ReGFR). AU patients, with or without ReGFR, had higher systolic and diastolic blood pressure, and higher total and LDL cholesterol (LDL-C) than patients with ReGFR alone or with neither AU nor ReGFR while HDL-C and triglycerides (TG) were increasingly worse in patients with AU alone, ReGFR alone and patients with both. HbA1c was significantly increased in AU patients without ReGFR. ReGFR patients, with or without AU, were older and had longer diabetes duration than patients with AU only and patients with neither AU nor ReGFR. Both AU and ReGFR were associated with higher prevalence of retinopathy and a history of macrovascular disease (including coronary heart disease, peripheral artery disease and stroke). However, “isolated” AU and ReGFR were associated with similar prevalence of macrovascular disease.

Conclusion: CV risk factor control, treatment and complications were quite different among type 2 diabetic patients with either AU, ReGFR or both. Of note, ReGFR patients without AU had a high burden of macrovascular disease, despite high treatment rates and moderately good risk factor control.

Clinical data stratified by presence of AU or ReGFR.

AU/ReGFR	No/No (N=2351, 46.1%)	Yes/No (N=1094, 21.5%)	No/Yes (N=812, 15.9%)	Yes/Yes (N=843, 16.5%)	All patients (N=5100)
Systolic blood pressure, mmHg	136 (134–138)	139* (138–141)	136 (134–138)	139* (137–141)	137 (136–138)
LDL-C, mmol/L	2.39 (2.33–2.44)	2.49* (2.42–2.56)	2.39 (2.31–2.47)	2.44* (2.36–2.52)	2.42 (2.38–2.46)
HDL-C, mmol/L	1.32 (1.30–1.34)	1.28* (1.25–1.30)	1.23* (1.19–1.26)	1.21** (1.18–1.24)	1.27 (1.26–1.29)
Fasting TG, mmol/L	1.51 (1.45–1.57)	1.69* (1.61–1.77)	1.68* (1.59–1.78)	1.81** (1.71–1.89)	1.62 (1.58–1.67)
HbA1c, %	7.56 (7.48–7.63)	7.90* (7.82–7.99)	7.60 (7.49–7.69)	7.67 (7.57–7.77)	7.66 (7.60–7.71)
Antihypertensive treatment, %	79.3 (77.0–81.6)	89.2* (87.0–90.9)	90.6* (88.0–92.8)	93.3** (90.9–95.0)	85.5 (83.8–87.0)
Lipid-lowering treatment, %	69.6 (67.0–72.1)	71.6 (68.2–74.5)	74.4* (70.8–77.8)	73.3* (69.7–76.8)	71.4 (69.3–73.5)
Retinopathy, %	24.8 (22.4–27.5)	39.4* (35.7–43.2)	33.1* (29.1–37.0)	45.2** (40.6–49.4)	32.6 (30.3–34.8)
Macrovascular disease, %	23.1 (21.2–25.3)	30.2* (27.1–33.6)	32.6* (28.9–36.3)	39.1** (35.1–42.9)	28.7 (27.0–30.4)

Mean or proportion (95% CI), adjusted for age, sex and diabetes duration.

* $P < 0.05$ vs. no AU

° $P < 0.05$ vs. no ReGFR

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Cardiovascular risk factors and prediction of albuminuria in patients with type 2 diabetes, a neural network analysis

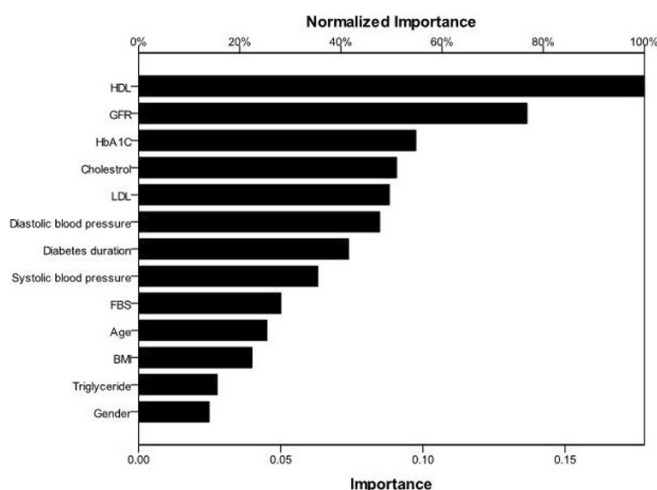
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Background and aims: Albuminuria is a sensitive marker of future cardiovascular events in patients with type 2 diabetes and healthy controls. Neural networks (NN) had attracted a great deal of attention for predicting outcome in biomedical researches. We aimed to assess the potential role of cardiovascular risk factors in the prediction of albuminuria in patients with T2DM, using a multilayer perception NN modeling.

Materials and methods: The study included 1162 patients with type 2 diabetes. Albuminuria was defined as urinary albumin excretion rate >30 mg/24 hour. The NN was employed for the prediction of normo/micro and macroalbuminuria. The patients were randomly classified into a: training (382/615; 62%), b: testing (169/615; 27.8%) and c: holdout (64/615; 10.2%) groups. NN uses learning algorithm to define the nonlinear mathematical transfer functions, to modify the synaptic weights of a network's processing units in an orderly fashion to attain the desired outcome prediction. Hence the model, which is extracted from the training group, is tested 3 times and the importance of each variable is checked by different weight and functions. A total of 552 out of 1162 cases were not included for NN modeling. Input variables were, age, sex, duration of diabetes, systolic blood pressure, diastolic blood pressure, Glomerular filtration rate (GFR), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), fasting plasma glucose, HbA1C, cholesterol and triglyceride.

Results: The study included 690 female and 472 male, aged 19-94 years. Of all the studied patients, 817/1162 (70%) had <30 mg/24 h, 203/1162 (17%) had 30-300 mg/24 h and 142/1162 (12%) had >300 mg/24 h albuminuria. Patients with albuminuria were older, had longer diabetes duration and a higher systolic blood pressure, diastolic blood pressure, HbA1C, triglyceride and a lower GFR and HDL compared to patients with normoalbuminuria. The model included 6 hidden layers and 1 bias. Percent of correct prediction was 80% in the training group, 74% in the testing group, and 77% in the holdout group. Percent of incorrect predictions were 20% in the training group, 26% in the testing group and 23% in holdout group. HDL had the greatest importance in the prediction of micro/macro albuminuria in the designed model (Figure 1). We compared patients with and without albuminuria with different cut points of HDL. Cardiovascular risk factors were the same for patients with a serum HDL higher than 40 mg/dl: either with micro/macro albuminuria and/or normoalbuminuria. All the cardiovascular risk factors were worst in patients with albuminuria and a serum HDL level lower than 40 mg/dl: compared to patients with normoalbuminuria. GFR had the second importance in prediction of albuminuria.

Conclusion: Consistent with our previous study, with a mathematics-based risk factor-outcome model, we showed the significance and importance of HDL in the prediction of albuminuria.

**PS 096 Nephropathy: biomarkers**

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Angiogenin levels are elevated in patients with diabetes and microalbuminuria

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Background and aims: The vast increase of diabetes in the western world challenges us with macrovascular and microvascular complications of the disease. Angiogenin, a small polypeptide, is associated with various forms of cancer and a potent inducer of vascular growth. In this study we investigated angiogenin as a possible biomarker for early microangiopathy/endothelial dysfunction in patients with diabetes.

Materials and methods: We measured angiogenin in 127 patients with diabetes (70 female, mean age: 51.4±16.2 years, HbA1c: 8.1±1.7rel.%). Albuminuria (ALB) was measured in 24-hour urine collections three times. ALB was defined by a least two consistent measurements over a three-month period: 86 normo-ALB (Co), 23 micro-ALB (MiA), and 18 macro-ALB (MaA). Angiogenin and Vascular Endothelial Growth Factor (VEGF) were measured by an ELISA from R&D Systems (Minneapolis, MN), Interleukin-18 (IL-18) by an ELISA from Medical & Biological Laboratories (Naka-ku Nagoya, Japan). Data are presented as median (25., 75. percentile). Statistics included Mann-Whitney-U test, Kruskal-Wallis test, and Spearman-Rho correlation, as applicable.

Results: Angiogenin levels were significantly higher in patients with ALB (359 (263,534) vs 285 (201,348) ng/ml, p=0.004). In addition, IL-18 showed a significant increase (p=0.033) in those with ALB, whereas VEGF only showed a trend towards increase (p=0.070). Furthermore, Angiogenin was higher in patients with MiA compared to Co (p=0.03), but did not differ to MaA. The presence of retinopathy did not change angiogenin levels (p=0.898). Additionally, angiogenin levels were elevated in men (p=0.047). In spearman-rho correlation analysis angiogenin levels were significantly associated with ALB (R=0.224, p=0.013), age (R=0.235, p=0.009), HbA1c (R=0.184, p=0.046), body mass index (R=0.275, p=0.003), weight (R=0.308, p=0.001), HDL cholesterol (R=-0.305, p=0.001) and triglycerides (R=0.297, p=0.002).

Conclusion: (Vascular) growth factors might be involved in the reduced prognosis of patients with MiA and MaA. We found increased angiogenin levels in patients with diabetes and albuminuria, but no relation to retinopathy.

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NT-proBNP predicts progression of CKD in type 2 diabetes

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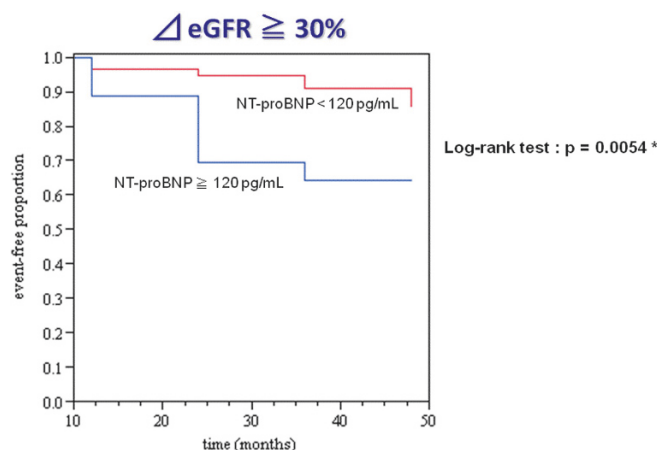
Background and aims: N-terminal proBNP (NT-proBNP) is postulated as a diagnostic and prognostic biomarker of heart failure and cardiovascular mortality in type 2 diabetes as well as in general population. We recently reported that NT-proBNP could be a marker of silent myocardial ischemia in type 2 diabetes. It is also known that BNP and NT-proBNP are elevated in CKD (chronic kidney disease) reflecting reduced glomerular filtration. It is possible that an additional mechanism might be present when considering the concept of cardiorenal continuum since major target organ of BNP secreted from heart is a kidney. In the present study, we tested whether NT-proBNP elevation could be a predictor of progression of CKD in type 2 diabetes with near normal kidney function.

Materials and methods: We consecutively recruited 109 patients (male 79, age 62.7±0.9, HbA1c 8.8±0.2, Cr 0.90±0.02mg/dl) with CKD stage 1 and 2. Serum NT-proBNP measurement (Roche) and ultrasonographic echocardiogram were performed and subjects were followed up to 5 years (median 44 months). Primary endpoints were defined as 1) renal: 30% decrease of eGFR due to low baseline Cr level and relatively short observation period and 2) CVD: cardiovascular disease (CVD) event and death.

Results: NT-proBNP was well correlated with left ventricular mass index and serum Cr. Baseline NT-proBNP levels were negatively associated with eGFR. During the follow-up period, fourteen patients reached the renal endpoint and had significantly higher baseline NT-proBNP than those who maintained eGFR (169 vs. 52 pg/ml, p<0.05). Patients were stratified into 2 groups

by NT-proBNP concentrations above and below the optimal cut-off points calculated by ROC analysis. Survival curves were analyzed using the Kaplan Meier calculation, and significantly higher proportions of subjects with NT-proBNP above 120 pg/ml at baseline had progressive decline of eGFR (Log rank test: $p=0.0054$). During the follow up period, eighteen patients had new incidences of CVD or death, namely CVD events. Mean levels of NT-proBNP were 78 and 43 pg/ml for those with and without new events, and these were significantly lower than that of the subjects with past CVD history ($p<0.05$). Subjects with NT-proBNP level above 60 pg/ml had significantly higher risks of CVD and death than those below 60 pg/ml (HR 3.77, $p=0.025$).

Conclusion: Both cardiac and renal functions are reflected by minimal elevations of NT-proBNP and it also predicted not only future CVD event or death as well as CKD progression in type 2 diabetes. As CKD is a major risk factor for CVD in diabetes, NT-proBNP might be one of key molecules linking kidney with CVD and eventual mortality. It is possible to stratify diabetic patients at high risk by simple measurement of NT-proBNP which is reproducible and inexpensive.



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RBP4 has an early marker of renal dysfunction in diabetic patients

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Background and aims: Fat tissue is viewed as an active endocrine organ with a high metabolic activity. Adipocytes produces and secretes several adipocytokines that play an important role in the metabolic control of diabetes and obesity. Retinol binding protein type 4 (RBP4) is produced by adipocytes and macrophages.

Materials and methods: Between December of 2008 and October of 2010, 309 patients were recruited with mean age 51 ± 13.5 years. They were divided in two groups: obese nondiabetic ($n = 133$) and diabetics ($n = 177$). The patients underwent anthropometric examination, with the determination of waist circumference, BMI and fat tissue using electric bioimpedance. The serum concentration of RBP4 was measured by nephelometry. Statistical analysis was made using the software SPSS vs. 17.

Results: Diabetics had higher urea, creatinine, triglycerides, alanine aminotransferase and gamma glutamyl transpeptidase values (7.2 ± 4.7 vs. 5.6 ± 1.6 mmol / L; 77.2 ± 30 vs. 69.6 ± 18.6 mmol / L; 1.8 ± 1.1 vs. 1.5 ± 0.7 mmol / L; 44.6 ± 24.7 vs. 32.3 ± 27.8 U/L; 51.9 ± 57.7 vs. 33.9 ± 45.6 U / L, $p < 0.05$) and lower HDL-C (1.2 ± 0.4 vs. 1.6 ± 0.4 mmol / L, $p < 0.05$) in comparison with the group of obese. In both groups we found a positive correlation between BMI and body fat mass with CRP ($r = 0.294$ vs. $r = 0.381$, $r = 0.384$ vs. $r = 0.398$, $p < 0.05$). Diabetic patients had higher values of RBP4 in comparison with the group of obese patients (53.5 ± 15.9 vs. 46.9 ± 17.8 mg/dl). Positive correlations were found with the urea and creatinine in both groups ($r = 0.449$ vs. $r = 0.498$, $r = 0.444$ vs. $r = 0.62$, $p < 0.01$). Positive correlation with 24 hours proteinuria was observed in diabetic patients ($r = 0.59$, $p < 0.01$) and in obese patients there was a positive correlation with triglycerides ($r = 0.374$, $p < 0.01$). No correlations were found with the anthropometric parameters.

Conclusion: This study highlights the role of adipocytokine in the metabolic profile: RBP4 is an excellent early marker of renal dysfunction. In the future it can be used to assess microvascular complications in type 2 diabetic patients.

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Insulin effect on agonists and antagonists of nitric oxide synthesis in type 2 diabetes mellitus with nephropathy

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Background and aims: The synthesis of nitric oxide (NO) is stimulated by insulin both *in vitro* and *in vivo*, and it is modulated by precursor substrates (like arginine and homoarginine), by competitive inhibitors of NO synthase (ADMA: asymmetric dimethylarginine), as well as by inhibitors of intracellular arginine transport (SDMA: symmetric dimethylarginine; and ADMA itself). In Type 2 Diabetes Mellitus (T2DM) we have previously demonstrated a reduced NO synthesis from arginine both under basal and hyperinsulinemic conditions. However, the role of ADMA, SDMA and homoarginine, on NO synthesis *in vivo*, as well as their response to hyperinsulinemia, are not known.

Materials and methods: Eight patients with T2DM and diabetic nephropathy (age: 63 ± 3 yrs; BMI: 28.9 ± 0.8 kg/m²), and ten non diabetic control subjects (age: 52 ± 6 yrs; BMI: 26.4 ± 0.8 kg/m²), all males, were studied for 180' under basal conditions and for subsequent 180' with a euglycemic, hyperinsulinemic clamp, following an iv. primed-continuous infusion of L-[¹⁵N₂-guanidino]-arginine. In the final 60' of each experimental period, under steady-state conditions, we measured the fractional synthesis rate (FSR) of NO (expressed as the sum of nitrites and nitrates, NOx), ADMA, SDMA and homoarginine concentrations, and we the search for correlations among these parameters.

Results: In the T2DM patients, with respect to controls, we found increased basal SDMA concentrations (0.60 ± 0.07 vs. 0.46 ± 0.03 μM, $p < 0.05$), reduced homoarginine concentrations (1.63 ± 0.19 vs. 2.12 ± 0.17 μM, $p < 0.06$), and a reduction of both arginine/SDMA (138 ± 25 vs. 191 ± 14 , $p < 0.03$) and arginine/ADMA ratios (145 ± 10 vs. 179 ± 9 , $p < 0.025$). In contrast, ADMA (0.51 ± 0.02 μM) and arginine concentrations (74 ± 7 μM) were similar to control values (0.48 ± 0.02 and 86 ± 5 μM, respectively). Hyperinsulinemia decreased ($p < 0.001$) ADMA, SDMA, homoarginine, and arginine concentrations, as well as both the arginine/SDMA and the arginine/ADMA ratios, in both groups. However, the differences between the groups were maintained. Inverse correlations were found between NOx FSR and ADMA ($r = -0.43$, $p < 0.01$), as well as between NOx FSR and SDMA ($r = -0.53$, $p < 0.001$). Conversely, direct correlations were found between NOx FSR and the arginine/SDMA ratio ($r = 0.51$, $p < 0.002$), but not between NOx FSR and the arginine/ADMA ratio.

Conclusions: In T2DM, SDMA concentration is increased, whereas that of homoarginine, as well as the arginine/SDMA and arginine/ADMA ratios, direct modulators of NOS activity, are reduced. Acute hyperinsulinemia affects arginine-related metabolites as well as NOS activity. SDMA, as well as the arginine/SDMA ratio, are correlated with NOx FSR. SDMA levels are more closely correlated than those of ADMA with NO synthase activity *in vivo*.

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Serum concentrations and urine excretion of matrix metalloproteases (MMPs) and transforming growth factor-β (TGF-β) in patients with diabetic nephropathy

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Background and aims: Dysbalance between the production and degradation of extracellular matrix by MMPs, especially gelatinases (MMP-2, MMP-9) and TGF-β signalling cascades play a major role in the progression glomerular sclerosis and fibrotic lesion in diabetic nephropathy (DN). The aim of our study was to evaluate the levels of serum and urine gelatinases and TGF-β and their association with renal damage markers - albumin-to-creatinine ratio (ACR), glomerular filtration rate (GFR) and hemoglobin (Hb) in patients (pts) with DN.

Materials and methods: We investigated 40 diabetic pts (13 type 1 and 27 type 2). Their mean clinical data: age - 50.0 ± 16.4 years, diabetes duration - 17.1 ± 9.8 years, HbA_{1c} - 9.1 ± 2.3 %, Hb - 130.0 ± 20.0 g/l, GFR was calculated by MDRD formula - 75.6 ± 38.6 ml/min/1.73 m². Of these, 10 pts had normoalbuminuria (NAU), 15 pts had microalbuminuria (micro-AU), 15 pts had macroalbuminuria (macro-AU). Anemia was defined by criteria for chronic kidney disease pts and WHO classification. The prevalence of anemia was 37.4%. We measured MMP-2, MMP-9 and TGF-β in serum (MMP-2, MMP-

9_{α} , TGF- β_1) and urine (MMP-2, MMP-9, TGF- β_1) by ELISA technique. The urine values were standardized to urine creatinine ratio. Pts with GFR<15 ml/min/1.73 m² were not included.

Results: As shown in Table 1 (* $p<0.05$; ** $p<0.01$; *** $p<0.001$ between NAU pts and other groups), the levels of serum and urine MMP-2 significantly increased in accordance with elevation of albuminuria degree and had a direct correlation with ACR (MMP-2_s, $r=0.69$; $p<0.001$; MMP-2_u, $r=0.76$; $p<0.001$). No differences were observed in MMP-9_s and TGF- β_1 levels in pts with elevated proteinuria, although urine excretion of MMP-9_u was higher in macro-AU pts and also was associated with ACR ($r=0.44$; $p<0.01$); TGF- β_1 was significantly lower in NAU pts. We found correlation of with GFR MMP-2_s ($r=-0.47$; $p<0.01$) MMP-2_u GFR ($r=-0.71$; $p<0.001$) TGF- β_1 ($r=-0.51$; $p<0.05$). The pts with anemia demonstrated higher levels of MMP-2_s (342.0 ± 74.6 vs 253.9 ± 69.0 ng/ml; $p<0.01$) as a MMP-2_u (1.5 ± 2.1 vs 0.4 ± 1.1 mcg/mmol; $p<0.01$). The measured parameters significantly correlated with Hb: MMP-2_s ($r=-0.69$; $p<0.001$); MMP-2_u ($r=-0.75$; $p<0.0001$); MMP-9_u ($r=-0.40$; $p<0.05$), TGF- β_1 ($r=-0.66$; $p<0.001$). Regression analysis of the above listed clinical data showed that they are significant predictors of MMP-2_s (ACR (beta=0.44), GFR (beta=-0.46), Hb (beta=-0.66)), MMP-2_u (ACR (beta=-0.48), GFR (beta=-0.56)) and TGF- β_1 (GFR (beta=-0.43), Hb (beta=-0.55)).

Conclusion: Elevation of serum concentration of MMP-2 in albuminuric pts may presumably displays their excess production in the kidney. Increasing the urine excretion of gelatinases, especially MMP-2 and TGF- β in advanced albuminuria and their association with signs of renal failure (GFR), and anemia (Hb) could more accurately reflect development of renal interstitial fibrosis.

Serum and urine levels of MMP-2, MMP-9 and TGF- β in diabetic patients according to albuminuria

Parameters	NAU pts	Micro-AU pts	Macro-AU pts
MMP-2, (ng/ml)	202.9 \pm 32.2	294.7 \pm 70.5***	335.2 \pm 75.4***
MMP-9, (ng/ml)	461.3 \pm 325.5	358.7 \pm 276.6	339.4 \pm 182.6
TGF- β_1 (ng/ml)	107.0 \pm 91.1	122.6 \pm 70.8	86.7 \pm 47.3
MMP-2 _u (mcg/mmol)	0.03 \pm 0.01	0.85 \pm 1.71*	1.32 \pm 2.04***
MMP-9 _u (mcg/mmol)	0.02 \pm 0.03	0.15 \pm 0.43	0.35 \pm 1.18*
TGF- β_1 (mcg/mmol)	0.33 \pm 0.29	0.73 \pm 0.67	0.90 \pm 0.42**

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Urinary excretion of matrix metalloproteinases and their inhibitors is related to albuminuria and renal fibrosis in type 1 diabetic patients

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Background and aims: Matrix metalloproteinases (MMPs) and metalloproteinase inhibitors play a principal role in extracellular matrix remodeling in renal glomeruli and interstitium and, therefore, is involved in pathogenesis of diabetic nephropathy (DN). The question remains whether urinary MMPs/MMP inhibitors could be the early markers of glomerular and interstitial fibrosis in diabetes. Thus, the aim of our study was to assess the relation between urinary excretion of MMPs and MMP inhibitors, albuminuria and the signs of renal fibrosis in type 1 diabetic patients.

Materials and methods: 64 patients with type 1 diabetes were examined, including 25 normoalbuminuric (group DN0), 30 microalbuminuric (group DN1) and 9 macroalbuminuric ones (group DN2). Urinary excretion of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinases-9 (MMP-9), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), tissue inhibitor of matrix metalloproteinases-2 (TIMP-2), plasminogen activator inhibitor-1 (PAI-1) and type IV collagen was determined by ELISA and compared to control (10 healthy subjects, group C). Fractional mesangial and interstitial volume was estimated in kidney biopsy specimens in 7 DN0- and 14 DN1-patients.

Results: (median, percentile 25-75). Urinary excretion (pg/ μ mol creatinine) of investigated MMPs, MMP inhibitors and type IV collagen is shown in the table.

Group	MMP-2	MMP-9	TIMP-1	TIMP-2	PAI-1	Type IV collagen
C	4.1 (2.5-6.0)	0.33 (0.22-0.70)	245 (39-973)	28.1 (12.8-56.6)	3.2 (2.3-6.2)	2.9 (2.2-4.3)
DN0	4.6 (2.5-7.5)	0.6 (0.34-1.73)	608 (22-1543)	30.5 (11.1-42.7)	5.3 (3.1-6.8)	3.6 (3.1-5.3)
DN1	6.0 (3.5-10.5)	0.87 (0.43-8.52)*	801 (32-1475)	31.8 (11.4-47.5)	7.0 (3.6-12.7)*	5.1 (2.8-7.8)*
DN2	16.1 (9.3-17.0)*	1.07 (0.50-1.45)	3104 (2233-5782)*	45.6 (43.5-54.2)	10.3 (6.1-19.3)*	15.2 (9.6-17.0)*

* $p<0.05$ vs. group C.

All parameters correlated positively with albuminuria (MMP-2: $r=0.71$; MMP-9: $r=0.29$; TIMP-1: 0.56 , TIMP-2: $r=0.57$; PAI-1: 0.41 ; all $p<0.05$). Significant correlations were found between excretion of type IV collagen and MMP inhibitors: TIMP-1 ($r=0.42$), TIMP-2 ($r=0.33$), PAI-1 ($r=0.39$). The increased TIMP-1 excretion was related to mesangial expansion ($r=0.44$), meanwhile TIMP-2 and PAI-1 correlated positively with the volume of interstitium ($r=0.39$ and $r=0.54$ respectively).

Conclusion: In type 1 diabetic patients increased urinary excretion of MMPs and MMP inhibitors is associated with albuminuria and the signs of glomerular and interstitial fibrosis.

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Urinary sulphate excretion is a predictor for progression of diabetic nephropathy

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Background and aims: Recently Hydrogen sulphide (H₂S) has emerged as a potentially important mediator of cardiovascular homeostasis and cytoprotection. H₂S is in part produced in the vasculature from L-cysteine where it mediates smooth muscle relaxation and subsequent vasodilatation. H₂S is converted to sulfite in the mitochondria, and then further oxidized to thio-sulphate and sulphate. The sulphates are finally excreted in the urine. It has been proposed that the biosynthesis of H₂S is reduced due to diabetes-related endothelial dysfunction. There is also some evidence that H₂S is the primary vessel-relaxing agent for small blood vessels. We have therefore measured the urinary excretion of sulphate in patients with type 1 diabetes (T1D) and diabetic nephropathy (DN) in order to evaluate if the sulphate excretion can predict progression of nephropathy.

Materials and methods: The study was a post hoc study of a prospective, randomized, unmasked, controlled trial. Originally 82 T1D patients with progressive DN were followed for 4 years, comparing the effects of a low-protein diet (0.6g/kg/day) with a usual-protein diet on decline in GFR (plasma clearance of ⁵¹Cr-EDTA) and development of ESRD or death. Sulphate excretion was measured by ion exchange chromatography in 24h urine at baseline, 2 years and 4 years. Data on sulphate was available for 65 of the subjects.

Results: There were 43 men and at baseline age [mean (SD)] was 40.1 (7.8) years. Baseline GFR was 71 (31) ml/min/1.73m². Baseline sulphate excretion [geometric mean (2.5-97.5% estimated centiles (EC))] was 10.0 (2.8-35.2) mmol/day. During follow-up decline in GFR was 3.8 (3.0) ml/min/year. Baseline log u-sulphate was associated with logAER ($p=0.011$, $r=0.313$), protein intake ($p<0.001$, $r=0.632$) and age at baseline ($p=0.001$, $r=-0.392$). It was not associated with baseline values for; GFR, HbA_{1c}, BP, cholesterol, assigned diet group, sex, tobacco use, use of antihypertensive medication or presence of cardiovascular disease at baseline. Baseline log u-sulphate excretion showed a significant negative association with the rate of decline in GFR ($p=0.015$, $r=-0.3$). When adjusted for diet, log AER and systolic BP at baseline, the correlation between sulphate and Δ GFR was even stronger ($p<0.001$, adj $r^2=0.32$). The same model without sulphate was weaker ($p=0.011$, adj $r^2=0.12$). During the study period sulphate excretion [geometric mean, (2.5-97.5% EC)] was 12.0 (5.0-28.8) mmol/day. Mean log u-sulphate during the study period was associated to the rate of decline GFR ($p=0.001$, $r=-0.402$), mean protein intake ($p<0.001$, $r=0.709$) and s-cholesterol ($p=0.034$, $r=-0.264$), but not to follow up values of BP, HbA_{1c}, logAER and HDL. The association was strong ($p<0.001$, adj $r^2=0.42$) when adjusted for mean values during follow-up of BP, logAER, HbA_{1c} and serum cholesterol (parameters found as independent

variables in the original study). The same model without sulphate was inferior ($p < 0.001$, adj $r^2 = 0.30$). We found similar results when we adjusted for assigned diet group and dietary protein intake (both $p < 0.001$, adj $r^2 = 0.41$).

Conclusion: We found that high urinary sulphate excretion is associated with slower rate of decline in GFR in diabetic nephropathy during four years of follow up, independent of known progression promoters.

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High urinary sodium excretion and association with albumin excretion in patients with type 2 diabetes mellitus

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Background and aims: Urinary sodium excretion, as a measure of sodium intake, has been associated to urinary albumin excretion in population based studies. Guidelines for clinical diabetes care recommend monitoring and treatment of albuminuria and advice dietary sodium restriction (100 mmol per day) for patients with diabetes, however recommendations for the monitoring of urinary sodium excretion are lacking. In this study we examined the sodium excretion per day in a cohort of patients with type 2 diabetes, and evaluated the relationship between sodium excretion and albumin excretion.

Materials and methods: Between May 2009 and December 2010 consecutive patients with type 2 diabetes attended to the outpatient clinic for their yearly comprehensive diabetes investigation were included. Clinical data including blood pressure, BMI, smoking status, HbA1c and 24 hour urinary sodium, potassium and albumin excretion, were evaluated and registered in a standardized way. Data are presented as medians with interquartile range (IQR). The cohort was divided into quintiles according to sodium excretion. Chi-square and Kruskal Wallis tests were performed.

Results: A total of 983 patients (52% men) with median age 63 (range 25-91) years were included. Median duration of diabetes was 11 (range 0-51) years, and HbA1c 54 (46-63) mM/M. Twelve patients did not collect 24 hour urine and were excluded. Urinary sodium excretion per day was ≤ 114 mmol $24h^{-1}$ in the 1st quintile and ≥ 251 mmol $24h^{-1}$ in the 5th quintile. Only in the 1st quintile the sodium excretion was in the range as expected with recommended sodium restriction. Median urinary albumin excretion was 11 (4-34) mg $24h^{-1}$ in the 1st versus 32 (11-84) mg $24h^{-1}$ in the 5th (p for trend < 0.001). We observed a positive significant association between sodium excretion and BMI, male gender, creatinin clearance and potassium excretion and a negative association with age (p for trend for these variables < 0.001). No difference was found for blood pressure, smoking, diabetes duration and complications, HbA1c, uric acid, and type or number of antihypertensives. The percentage of patients with a sodium excretion ≥ 150 mmol $24h^{-1}$ increased with the severity of albuminuria from 56% in patients with normal levels to 67% and 80% in patients with microalbuminuria and proteinuria respectively ($P < 0.001$).

Conclusion: Sodium excretion per day was high in many patients and was positively related to the level of albuminuria. Since a beneficial effect of salt restriction is likely in patients with an elevated urinary albumin excretion, we suggest to measure 24 hour urinary sodium excretion to monitor salt intake and guide treatment.

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Increased urinary excretion of cystatin C predict renal impairment in type 2 diabetes

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Background and aims: Although albuminuria is one of the most conventional markers for the progression of diabetic nephropathy, it is casting doubt on the legitimacy of this marker for early detection of diabetic nephropathy. The aim of this study was to evaluate clinical usefulness of urine cystatin C (Ucyst C) levels in predicting renal impairment ($eGFR < 60$ mL/min/1.73m²).

Materials and methods: We studied 305 patients with type 2 diabetes who measured Ucyst C at baseline in Pusan National University between 2008 and 2010. We excluded 18 patients with $eGFR < 30$ mL/min/1.73m² at baseline.

Results: Of the 287 patients, 54 (18.8%) showed renal impairment ($eGFR < 60$ mL/min/1.73m²) during follow-up period. The median follow up period was 20 months. Ucyst C was significantly associated with renal impairment during follow-up period in univariate analysis ($P < 0.001$). A Cox proportional hazard regression model was used to analyze various factors for renal impairment in multivariate analysis. The factors that can predict renal impairment during follow-up was high Ucyst C level (Ucyst C > 0.1 mg/L; HR=2.706, 95% CI 1.471-4.977; $P=0.001$), age (HR=1.047, 95% CI 1.013-1.082; $P=0.006$) and SBP (HR=1.025, 95% CI 1.002-1.048; $P=0.029$). When we analyzed data of 264 patients with normo- and microalbuminuria, high Ucyst C level was the only factor to predict renal impairment during follow-up (HR=2.692, 95% CI; 1.320-5.489; $P=0.006$).

Conclusion: Ucyst C could be a useful marker for predicting renal impairment independently of other factors including albuminuria in type 2 diabetic patients.

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Increased urinary excretion of myo-inositol and D-chiro-inositol in diabetes occurs independently of hyperglycaemia in the isolated perfused kidney

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Background and aims: Abnormal urinary clearance of the inositol sugars, myo-inositol (MI) and D-chiro-inositol (DCI), occurs in both diabetes mellitus and in polycystic ovary syndrome (PCOS). D-chiro-inositol is a putative insulin mimetic that lowers hyperglycemia in diabetes and in PCOS its increased urinary excretion is inversely correlated with insulin sensitivity. Currently, the mechanisms underlying increased urinary inositol excretion are unknown although it has been presumed that hyperglycemia is a major contributing factor. This conclusion is based in part on the inhibitory effect of high glucose on inositol uptake in cultured proximal tubular-derived cells via competition with inositol transporters. However, in vitro cultures of single-type cells do not represent the physiological function of the kidney where urinary excretion is regulated by the interplay of renal re-absorption, filtration, and excretion. Also, hyperglycemia does not explain the same phenomenon in PCOS which is characterised by a hyperinsulinemic state. The aim of the present study was to therefore investigate the significance of high glucose ambience on inositol excretion in diabetes using an intact physiological kidney system.

Materials and methods: The isolated perfused kidney was established, validated, and used to measure inositol excretion in kidneys from rats that had been diabetic for 4-weeks from streptozotocin injection. Kidneys isolated from saline-injected rats were used as a non-diabetic control. Kidney viability was assessed using several criteria including glomerular filtration rate (GFR), glucose re-absorption, sodium ion re-absorption, urinary flow rate and perfusion pressure. The perfusion system comprised a single pass pump-driven force perfusion containing 50μM MI and 5μM DCI. Diabetic and non-diabetic kidneys were perfused with perfusate containing either 5mM or 20mM glucose and stabilised for 40 minutes prior to collection of urine samples. MI and DCI concentrations were determined using GC/MS utilising deuterated myo-inositol as an internal standard.

Results: The isolated perfused kidney system successfully preserved the functionality of the kidney. Compared to the non-diabetic controls, diabetic kidneys perfused with either 5mM or 20mM glucose displayed impaired functionality with lower GFR and Na⁺ re-absorption. In diabetic kidneys perfused with 5mM glucose, the GFR-normalised urinary excretion of MI and DCI were increased by 1.4-fold and 4-fold ($p < 0.001$), respectively, compared to control kidneys. In both non-diabetic and diabetic kidneys perfused with 20mM glucose, there was a further potentiated increase in DCI excretion by approximately 1.4-fold ($p < 0.05$).

Conclusion: The primary cause of excessive urinary excretion of MI and DCI is due to a fundamental impairment in kidney function as a result of prolonged exposure to diabetes. It is also primarily a renal phenomenon as opposed to any systemic abnormality in inositol metabolism. The effects of hyperglycemia are secondary to this impairment in kidney function and were selectively pronounced for DCI. The selective 4-fold increase in DCI urinary excretion in the diabetic kidney is consistent with clinical observations in diabetes. As the increased DCI excretion also occurred under normoglycemic conditions, it is possible that a similar impairment in kidney function contributes to the abnormal urinary excretion of DCI in PCOS.

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PS 097 Nephropathy: treatment

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Trends in cost of prescription medication by stages of diabetic nephropathy in patients with type 1 diabetes between 1995 and 2005

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Background and aims: While previous studies have established the relationship between medication cost and kidney failure, typically the cost with respect to earlier stages of the disease development are not considered. Using longitudinal data from a large nationwide prospective cohort of adult patients with type 1 diabetes, we were able to estimate the outpatient prescription medication use and costs by different stages of diabetic nephropathy.

Materials and methods: The Finnish Diabetic Nephropathy Study (FinnDiane) data (N=1 670) were linked to the Drug Prescription Register. Based on their urinary albumin excretion rate patients were divided into three nephropathy groups: normoalbuminuria, microalbuminuria and macroalbuminuria. The fourth group consisted of patients with a kidney transplant. Moreover, data on progression of renal disease were verified from baseline and follow-up visits as well as medical files, until 31 December 2005 and all non-progressors were included in this analysis. Costs were inflated to 2009 euro levels by using the Consumer Price Index. The mixed linear models were used to evaluate the annual costs during 1995–2005. In order to obtain information on co-morbidities we also linked the FinnDiane data with the Hospital Discharge Register and the Central Drug Register. Costs were adjusted for age, sex, contributing years, BMI, total insulin dose/day, co-morbidities and years with kidney transplant in the final multivariate models.

Results: After adjustment, differences were observed between different stages of nephropathy ($p=0.02$). Interaction between year and nephropathy stage was significant ($p<.0001$), which manifested as different cost profiles between groups. However, no differences were observed with respect to annual costs ($p=0.4$) and cost profiles ($p=0.9$) between normo- and microalbuminuria groups. Therefore, these two groups were pooled in the further analysis. Adjusted costs between 1995 and 2005 increased linearly in normo- and microalbuminuria by 37% (from €1226 to €1681) and in macroalbuminuria groups by 64% (from €1286 to €2109). Although the annual adjusted costs per patient were the highest in patients with kidney transplant, the costs decreased by 45% (from €8635 to €5973) during this period. In 1995 the adjusted cost difference between pooled (normo- and microalbuminuria) and macroalbuminuria groups was only 5% (€60), whereas in 2005 it increased to 26% (€428). The difference between macroalbuminuria and kidney transplantation was 5.7-fold (€7349) in 1995. However, the gap decreased by time and the costs were 1.8 times lower (3864€) in 2005.

Conclusion: Progression from normo- and microalbuminuria to more severe stages of nephropathy had a strong impact on prescription medication costs in patients with type 1 diabetes. The increasing trend in the costs among normo-, micro- and macroalbuminuric patients suggests that new and more expensive drugs and strict evidence-based guidelines have had an effect on the costs. In contrast, the decrease in the costs for those with kidney transplants indicates that the costs for immunosuppressants have decreased probably due to expired patents, price competition and generic substitution.

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Risk of major renal events in people with type 2 diabetes: prediction models based on the ADVANCE study population

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Background and aims: Among patients with type 2 diabetes, the incidence of kidney disease continues to contribute to significant morbidity and mortality. However to date, insufficient data have been available to develop reliable risk prediction tools. We developed a risk prediction model for major renal

events in patients with diabetes using the data from the Action in Diabetes and Vascular Disease: Preterax and Diamicon Modified Release Controlled Evaluation (ADVANCE) trial.

Materials and methods: ADVANCE recruited 11,140 participants with type 2 diabetes aged ≥ 55 years, with a history of major macrovascular or microvascular disease or at least one other risk factor for vascular disease. Major renal events were defined as the composite of renal replacement therapy, renal death or doubling of serum creatinine to $\geq 200\mu\text{mol/l}$. The risk prediction model was developed using baseline characteristics in Cox proportional hazards regression with adjustment for randomised treatments (blood pressure lowering and glucose control interventions).

Results: During a median of 5 years follow up, 166 major renal events were recorded. Significant predictors for subsequent major renal event in the multivariable analysis were estimated GFR, urine albumin/creatinine ratio, systolic blood pressure, HbA1c, presence of diabetic retinopathy, male sex and level of formal education. When applied to the ADVANCE cohort, this model demonstrated good discrimination (area under the receiver operating characteristic curve, 0.83; 95% CI, 0.80–0.87) and excellent calibration (Hosmer-Lemeshow χ^2 statistic 8.37, df=9, $p=0.50$).

Conclusion: This simple model comprising 7 variables routinely assessed in clinical practice can accurately predict the risk of major renal outcomes in patients with type 2 diabetes. This model requires validation in an external cohort.

Clinical Trial Registration Number: NCT00145925

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Telmisartan attenuates diabetic nephropathy through anti-oxidative and anti-inflammatory actions via activation of peroxisome proliferator-activated receptor- γ

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Background and aims: Microinflammation contributes to the pathogenesis of diabetic nephropathy. We previously reported that pioglitazone, peroxisome proliferator-activated receptor (PPAR)- γ agonist, inhibits macrophage infiltration into kidney and exerts renoprotective effects via anti-inflammatory effects in diabetic rats. Telmisartan, which is an angiotensin receptor blocker, is reported to act as PPAR- γ partial agonist. The aim of this study is to investigate the renoprotective effects of telmisartan via activation of PPAR- γ .

Materials and methods: Five-week male SD rats were used in this study. Diabetes was induced by intravenous injection of streptozotocin at 65mg/kg body weight. Non diabetic control rats were administered with citrate buffer instead of streptozotocin. Diabetic rats were divided into three groups, and administered with each drug for 8 weeks after induction of diabetes: The first group (TEL) is administered with telmisartan 5mg/kg body weight daily by gavage, the second group (GW) is administered with telmisartan 5mg/kg and GW9662, selective PPAR- γ antagonist, 0.5mg/kg daily by gavage, and the last group (HYD) is administered with hydralazine 5mg/kg daily in drinking water (diabetic controls). AER, blood pressure, blood glucose level were measured at 2, 4, 8 weeks. We collected kidney, blood and urine samples at 8 weeks, and metabolic data, histology and morphometry were evaluated.

Results: No significant difference for the level of HbA1c was observed between the diabetic three groups. No significant difference for the level of blood pressure was observed between the diabetic three groups and non diabetic control group. AER at 8 weeks was significantly reduced in TEL compared with HYD (HYD $947\pm 148\mu\text{g/day}$ (Mean \pm SE) vs. TEL $438\pm 46.3\mu\text{g/day}$, $p=0.01$), and the effect of telmisartan was partially antagonized by administration of GW9662 (GW $736\pm 97.1\mu\text{g/day}$). Mesangial matrix expansion was observed in diabetic rats, however, the expansion was significantly suppressed in TEL compared with HYD ($p<0.05$), and the effect of telmisartan was partially antagonized by GW9662. Glomerular expression of ICAM-1 and type IV collagen was increased in diabetic rats, however, the expression was significantly suppressed in TEL compared with HYD (ICAM-1; $p<0.001$, type IV collagen; $p<0.05$), and the effect of telmisartan was partially antagonized by GW9662. Urinary excretion of 8-Hydroxydeoxyguanosine was increased in diabetic rats, however, the expression was significantly suppressed in TEL compared with HYD, and the effect of telmisartan was partially antagonized by GW9662. Gene expression of CD14 in renal cortex was significantly up-regulated in diabetic rats, however, the expansion was suppressed in TEL compared with HYD, and the effect of telmisartan was partially antagonized

by GW9662. The number of infiltrated macrophages into the glomeruli was increased in diabetic rats, however, the number was significantly decreased in TEL compared with HYD ($p<0.001$), and the effect of telmisartan was partially antagonized by GW9662. The same result was observed about sialoadhesin positive activated macrophages.

Conclusion: These results suggest that telmisartan exerts renoprotective effects through anti-oxidative and anti-inflammatory actions via activation of PPAR- γ in diabetic rats.

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Effectiveness of angiotensin II receptor antagonists in a Dutch cohort of patients with type 2 diabetes (ZODIAC-14)

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Background and aims: Blockage of the renin-angiotensin system (RAS) is an important therapeutic option to treat hypertension and/or albuminuria in patients with type 2 diabetes mellitus (T2DM). In this current study, we investigated the effects of different ARBs on blood pressure and albuminuria after 1 year in a large cohort of patients with T2DM.

Materials and methods: In 2007, 24940 primary care patients with T2DM participated in the ZODIAC study, a prospective observational study. Of these patients, 4810 (19%) patients were on ARBs. One year follow-up data were available for 3912 patients, of whom 3678 (94%) still used an ARB. A general linear model (GLM) for univariate analyses was used to estimate effects of losartan, irbesartan and a combined group of other ARBs on systolic blood pressure (SBP) and albuminuria. Analyses were repeated in strata according to baseline hypertension (SBP <140 mmHg and SBP \geq 140 mmHg) and albuminuria (albumin creatinine ratio (ACR) \geq 2.5 mg/mmol in men and \geq 3.5 mg/mmol in women). Because of skewed distribution ACR was logarithmically transformed (LnACR). Age, gender, baseline SBP, baseline LnACR, presence of macrovascular complications, use of diuretics and insulin were entered as covariates. Bonferroni was used to adjust for multiple comparisons. **Results:** A total of 1863 patients were on losartan, 1292 on irbesartan and 1655 on another ARB. Data on blood pressure in 2007 and 2008 were missing in 3% and 1% of all patients respectively. For ACR the proportions were 31% and 15% respectively. Use of diuretics and insulin was (significantly) higher in patients on irbesartan. Presence of macrovascular complications was lowest in the losartan group and highest in the combined group. No relevant or significant differences were found between groups concerning age, sex, blood pressure, albuminuria, renal function and use of other antihypertensive or glucose lowering drugs. Mean LnACR values in 2007 were 0.57, 0.62 and 0.35 in the losartan, irbesartan and the combined group of other ARBs, respectively. Median ACR in 2007 and 2008 were 1.0 in all groups. Results are presented in table 1. We found a significantly higher ACR in patients with SBP <140 mmHg on Irbesartan vs. other ARBs.

Conclusion: In this observational study with one year follow-up, different ARBs were equivalent with respect to the effects on blood pressure and ACR, except for a significant difference between Irbesartan vs. other ARBs in ACR in patients with normal baseline SBP, although this difference is hardly relevant.

Table 1.

	All (n=4810)	SBP <140 mmHg (n=1650)	SBP \geq 140 mmHg (n=2994)	No albuminuria (n=2455)	Albuminuria (n=873)
Comparison SBP (mmHg)					
Losartan vs. Irbesartan	-0.54 (-2.52;1.43)	-0.52 (-3.39;2.35)	-0.57 (-3.20;2.07)	-0.11 (-2.41;2.19)	-1.94 (-5.82;1.95)
Losartan vs. other ARBs	1.27 (-0.61;3.15)	0.53 (-2.09;3.14)	1.60 (-0.95;4.15)	1.15 (-1.00;3.31)	1.52 (-2.32;5.35)
Irbesartan vs. other ARBs	1.81 (-0.21;3.84)	1.05 (-1.82;3.92)	2.16 (-0.56;4.89)	1.27 (-1.05;3.58)	3.45 (-0.72;7.62)
Comparison ACR(mg/mmol)					
Losartan vs. Irbesartan	0.98 (0.86;1.13)	0.85 (0.68;1.08)	1.05 (0.89;1.23)	1.06 (0.92;1.22)	0.84 (0.62;1.15)
Losartan vs. other ARBs	1.04 (0.91;1.19)	1.08 (0.88;1.34)	1.01 (0.86;1.19)	1.07 (0.93;1.22)	1.03 (0.76;1.39)
Irbesartan vs. other ARBs	1.06 (0.92;1.21)	1.27 (1.01;1.60)	0.96 (0.81;1.14)	1.01 (0.87;1.16)	1.22 (0.88;1.68)

Difference between groups (95% confidence interval). The results of the analyses with LnACR were back transformed to the ratio of the geometric means.

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Initial angiotensin receptor blockade-induced decrease in albuminuria predicts long term renal outcome in type 2 diabetic patients with microalbuminuria

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Background and aims: We aimed to investigate the individual impact of initial responses in urinary albumin excretion (UAE) and systolic blood pressure (SBP) to Angiotensin-Receptor-Blockade (ARB) treatment on long term renal outcome in patients with type 2 diabetes and microalbuminuria.

Materials and methods: In a post-hoc analysis of the IRMA-2 trial we first assessed the individual variability in UAE and SBP-response (0-6 months) in 531 subjects. Subsequently, we analyzed the individual effect of both response parameters on renal outcome defined as change in estimated glomerular filtration rate (eGFR) during 2 years of follow-up.

Results: The median reductions in UAE and SBP in the population were -18% and -11mmHg respectively. In Irbesartan treated patients, 85 patients (24%) had a robust (>median) reduction in UAE but not in SBP (discordant SBP-response) and 67 patients (19%) had a robust (>median) reduction in SBP but not in UAE (discordant UAE-response). The degree of reduction in UAE was independently associated with the rate of eGFR decline ($p=0.0037$). SBP showed a similar trend ($P=0.087$). The relation between a larger UAE-reduction and a slower rate of renal function decline was present in both the cohort with a SBP-change above and below the median.

Conclusion: Within an individual UAE response to ARB-therapy may be discordant from SBP-response. The initial change in UAE was independently associated with eGFR slope: the more UAE reduction the less eGFR decline, irrespective of the SBP-change. These results suggest that in microalbuminuric patients with type 2 diabetes, UAE should be monitored after initiation of therapy and a separate target for renoprotective therapy.

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1095

Effects of valsartan add-on treatment on aldosterone breakthrough in type 2 diabetic patients with hypertension receiving enalapril

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Background and aims: Angiotensin II receptor antagonists (ARB) are the first choice for treatment of hypertension and prevention of organ damage in diabetic patients with hypertension. One of the major effects of ARB is to decrease plasma aldosterone concentration (PAC) for cardiovascular protection. However, long-term ARB therapy is associated with increased PAC, which is known as 'aldosterone breakthrough'. Coadministration of ARB and angiotensin converting enzyme inhibitors (ACEI) is more effective for the treatment of hypertension than monotherapy of either in diabetic patients with hypertension. Nevertheless, little is known about the effect of coadministration of both agents on aldosterone breakthrough.

Materials and methods: This prospective randomized parallel-group study was designed to compare the effects of adding valsartan (ARB) to enalapril (ACEI) and doubling the dose of enalapril in type 2 diabetic patients whose

blood pressure did not reach the therapeutic goal (140/90 mmHg) by enalapril (5 mg/day) monotherapy. Fifty-four patients (63 ± 7 years old, 32 men and 22 women, serum creatinine levels < 1.5 mg/dL) already on enalapril 5 mg/day were assigned to receive either a double dose of enalapril (10 mg) or valsartan (80 mg) added to enalapril (5 mg) for 48 weeks. Plasma renin activity (PRA) and PAC were measured at 0, 12, 24 and 48 weeks.

Results: Both groups showed significant ($p < 0.05$) decreases in blood pressure during the treatment period from 148/94 at 0 week to 132/86 mmHg at 12 weeks in the enalapril double-dose group and from 150/95 at 0 week to 134/84 mmHg at 12 weeks in the valsartan add-on group). In the valsartan add-on group, PAC decreased from 86 ± 6 at 0 week to 65 ± 5 pg/mL at 12 weeks ($P < 0.05$) and maintained similar levels during therapy. However, in the enalapril double-dose group, PAC initially decreased from 84 ± 7 (0 week) to 65 ± 6 pg/mL ($P < 0.05$) at 12 weeks but returned to the baseline level (81 ± 7 pg/mL) at 24 weeks. PRA increased from 1.7 ± 0.5 (0 week) to 4.4 ± 1.0 ng/mL/h ($p < 0.01$, 12 week) in the valsartan add-on group and from 1.9 ± 0.4 (0 week) to 4.7 ± 1.2 ng/mL/h ($p < 0.01$, 24 week) in the enalapril double-dose group.

Conclusion: These findings suggest that adding valsartan to enalapril suppresses aldosterone breakthrough more effectively than increasing the dose of enalapril. Aldosterone increases cardiac fibrosis and promotes ventricular remodeling, which incurs high mortality in type 2 diabetic patients with hypertension. Thus, coadministration of ACEI and ARB may minimize these aldosterone-induced deleterious effects.

1096

Effects of valsartan on osteoprotegerin and receptor activator of nuclear factor kappa B ligand levels in patients with type 2 diabetes, systolic hypertension and microalbuminuria

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Background and aims: Osteoprotegerin (OPG) and Receptor Activator of Nuclear factor- κ B Ligand (RANKL) are important glycoproteins involved in bone homeostasis. The OPG/RANKL axis may play a role in cardiovascular disease (CVD). Recent studies have reported a positive correlation between OPG and aortic pulse wave velocity (Ao-PWV), an index of arterial stiffness and an independent marker of CVD. We have previously demonstrated that in patients with Type 2 Diabetes Mellitus (T2DM), systolic hypertension and microalbuminuria, treatment with Valsartan and Hydrochlorothiazide (VAL/HCTZ), as compared to Amlodipine (AML) significantly reduced Ao-PWV and albumin excretion rate (AER) independent of blood pressure control. Our objective was to evaluate whether the beneficial effect of VAL/HCTZ on Ao-PWV correlated with changes in OPG and RANKL serum levels.

Materials and methods: In a 24 week double-blind, parallel group study we evaluated the effects of VAL/HCTZ vs. AML on the primary outcome Ao-PWV, in 131 T2DM patients with systolic hypertension, raised AER and preserved renal function. HCTZ was added to VAL to ensure equivalent blood pressure control vs. AML. In this post-hoc study we measured serum OPG and RANKL levels in 125 (VAL/HCTZ $n=64$, AML $n=61$) subjects. Serum OPG and RANKL levels were determined by enzyme-linked immunosorbent assay from samples obtained at baseline and end of the study. Ao-PWV was measured by applanation tonometry. All measurements and statistical analyses were performed blinded to group allocation.

Results: There were no significant baseline differences between the two groups in age, gender, duration of diabetes, renal function, blood pressure measures and glycaemic control. Following 24 weeks, Ao-PWV was reduced to a significantly greater extent with VAL/HCTZ, mean (95%CI), -1.7 (-1.1 , -2.3) m/s as compared to AML, -0.5 (0.04 , -1.03) m/s, $p < 0.001$. At baseline, a linear regression model showed that neither OPG nor RANKL levels demonstrated a significant relationship with Ao-PWV. The differential effects of treatment on Ao-PWV did not correlate with changes in serum OPG and RANKL levels or their ratio. No significant effect of treatment (VAL/HCTZ vs. AML) was observed on OPG and RANKL serum levels, and their ratio. Based on multivariate regression analysis, OPG and RANKL changes (Final - Baseline) only associated with gender ($p=0.014$ for OPG, $p=0.06$ for RANKL), while OPG/RANKL ratio change (Final - Baseline) associated with log C-reactive protein ($p=0.007$). However, OPG, RANKL and OPG/RANKL ratio did not associate with treatment type and any other baseline characteristics.

Conclusion: The observed significant reduction in Ao-PWV by Valsartan is not associated with changes OPG or RANKL levels. These results suggest that

OPG and RANKL serum levels are unlikely to mediate the blood pressure independent cardiovascular protective properties of angiotensin receptor blockers in T2DM.

Clinical Trial Registration Number: NCT00171561

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Glycosylation gap is influenced by Irbesartan treatment in patients with type 2 diabetes and microalbuminuria

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Background and aims: Inhibition of the renin-angiotensin-aldosterone system by the use of angiotensin II receptor blockers (ARBs) and angiotensin converting enzyme inhibitors is considered a first-line treatment modality for diabetic patients with microalbuminuria. Irbesartan is a potent and selective angiotensin II subtype 1 receptor ARB used in the treatment of diabetic nephropathy. The aim of this study was to assess the effect of Irbesartan on the glycosylation gap - the difference between measured HbA_{1c} and that computed from measured plasma fructosamine content.

Materials and methods: Fifty-two hypertensive type 2 diabetic patients with microalbuminuria (41 males) were recruited from our center. At inclusion, antihypertensive treatment was discontinued and replaced with bendroflumethiazide, 5 mg once daily, and following 2-months washout (baseline), patients were treated with Irbesartan, either 300 mg (17 patients), 600 mg (17 patients), or 900 mg (18 patients) once daily, for 2 months. HbA_{1c} and plasma protein fructosamine content were recorded and the glycosylation gap deduced at baseline and post-Irbesartan treatment. Plasma protein fructosamine content was determined by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry of exhaustive enzymatic digests.

Results: Irbesartan treatment produced a negative shift in the glycosylation gap: baseline $0.00 \pm 1.15\%$ versus $-3.24 \pm 1.15\%$ ($P < 0.001$; paired t-test). There was no difference in glycosylation gap between treatment groups at baseline but there was after Irbesartan treatment ($P < 0.01$, one-way ANOVA), attributed to a greater change in glycosylation gap by 600 mg o.d. Irbesartan treatment than for 300 and 900 mg o.d. doses. Glycosylation gap by Irbesartan dose was: 300 mg o.d., $-3.18 \pm 1.18\%$; 600 mg o.d., $-3.98 \pm 1.04\%$ ($P < 0.05$, with respect to 300 mg o.d.); and 900 mg o.d., $-2.61 \pm 1.19\%$ ($P < 0.001$, with respect to 600 mg o.d., t-test). The marked negative shift of the glycosylation gap with Irbesartan treatment was due to increase in plasma fructosamine, probably linked to decreased clearance of glycated albumin when the glomerular filter is tightened with Irbesartan treatment.

Conclusion: Irbesartan treatment produced a negative shift of glycosylation gap in patients with type 2 diabetes and microalbuminuria. This is the first demonstration of renin-angiotensin-aldosterone system directed treatment influencing the glycosylation gap.

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Impact of glycaemic control on the effect of direct renin inhibition in the AVOID study

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Background and aims: Hyperglycaemia induces development and progression of microvascular complications in diabetes. Furthermore, a new direct link between high glucose levels and intrarenal renin-angiotensin activation has been demonstrated. This post hoc analysis assessed the influence of baseline glycaemic control (HbA_{1c}) on the reduction of albuminuria of aliskiren or placebo added to losartan in the Aliskiren in the Evaluation of Proteinuria In Diabetes (AVOID) study.

Materials and methods: In AVOID, 599 patients aged 18–85 years with type 2 diabetes, hypertension and nephropathy received 6 months' aliskiren (150 mg force titrated to 300 mg after 3 months) or placebo added to losartan 100 mg and optimal antihypertensive therapy. Key exclusion criteria were non-diabetic kidney disease, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m² and serum potassium >5.1 mmol/L. Changes in urinary albumin creatinine ratio (UACR) at end of study were assessed by tertiles of baseline HbA_{1c} levels.

Results: Patient baseline characteristics showed no notable differences between HbA_{1c} subgroups. Patients were divided into tertiles of HbA_{1c} (<7.1%, ≥7.1–<8.4% and ≥8.4% respectively). Whereas mean HbA_{1c} did not change during the study in patients receiving aliskiren (baseline 8.0%, endpoint 8.0%), there was a 0.2% increase in mean HbA_{1c} in the placebo group (baseline 7.9%, endpoint 8.1%; $p = 0.012$ vs aliskiren). In the overall population, adding aliskiren to losartan reduced UACR by 20% compared to placebo ($p < 0.001$). The antiproteinuric effect of aliskiren was consistent across subgroups of baseline glycemic control, with the largest effect (tertile 1 vs. 3, $p=0.052$) in the tertile with baseline HbA_{1c} ≥8.4% (Table). In linear regression analysis there was no association between baseline HbA_{1c} and reduction in UACR in the aliskiren treated group.

Conclusion: This post hoc analysis of the AVOID study suggests that renin inhibition with aliskiren 300 mg once daily added to losartan 100 mg once daily plus optimal antihypertensive therapy provides reductions in UACR that are efficacious in all, but particularly in poorly controlled diabetic patients.

HbA _{1c} tertile	Aliskiren UACR ratio (95% CI) for (end of study:baseline)	Placebo UACR ratio (95% CI) for (end of study:baseline))	Geometric mean ratio (95% CI) for UACR (aliskiren:placebo)	p-value
HbA _{1c} <7.1%	0.92 (0.78–1.10)	1.07 (0.92–1.24)	0.86 (0.69–1.08)	0.196
HbA _{1c} ≥7.1% and <8.4%	0.81 (0.69–0.95)	0.96 (0.84–1.09)	0.85 (0.69–1.03)	0.104
HbA _{1c} ≥8.4%	0.73 (0.61–0.87)	0.99 (0.81–1.22)	0.73 (0.56–0.95)	0.021

Clinical Trial Registration Number: NCT00097955

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1099

Spirolactone diminishes urinary albumin excretion in type 1 diabetic patients with microalbuminuria: a randomised placebo-controlled crossover study

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Background: It has been shown that adding the aldosterone receptor blocker spironolactone to standard renoprotective treatment, including renin-angiotensin-aldosterone system (RAAS) blockade in diabetic nephropathy, provides additional antiproteinuric effects. We examined the antiproteinuric effect of spironolactone on markers of glomerular and tubular damage in type 1 diabetic patients with persistent microalbuminuria.

Material and methods: We performed a randomized, placebo controlled, double blind, crossover study in 21 type 1 diabetic patients with microalbuminuria using spironolactone 25 mg or placebo once daily for 60 days, added to optimal antihypertensive treatment including RAAS blockade in maximal recommended doses. After both treatment periods, endpoints were evaluated: urinary(u)-albumin excretion/24hour(h), 24h blood pressure, glomerular filtration rate (GFR) and markers of tubular damage: urinary liver-type fatty-acid binding protein (LFABP), neutrophil gelatinase associated lipocalin (NGAL) and kidney injury molecule 1 (KIM1).

Results: All patients completed the study. During spironolactone treatment, u-albumin excretion was reduced by 60% (21–80) from 90 mg/24h to 35mg/24h when compared with placebo ($p=0.01$). Blood pressure (24h) did not change during spironolactone treatment ($p>0.2$ for all comparisons), and GFR decreased from 78(8) to 72(6) mL/min/1.73m² ($p=0.003$). The tubular markers u-LFABP, u-NGAL and u-KIM1 did not change during treatment ($p>0.3$ for all comparisons). Treatment was well-tolerated, two patients developed severe hyperkalemia (plasma potassium=5.7 mmol/L), which were sufficiently treated with diuretics and dietary intervention.

Conclusions: We demonstrated that spironolactone treatment on top of recommended optimal renoprotective treatment in microalbuminuric type 1 diabetic patients, may offer additional renoprotection independent of effects on systemic blood pressure. Plasma potassium should be monitored carefully. Clinical Trial Registration Number: Eudra CT: 2008-004839-38

PS 098 Hypertension: epidemiology and treatment

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Blood pressure control in hypertensive patients with diabetes mellitus and impaired renal function in real life: results of the 3A registry

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Background: Blockade of the renin angiotensin aldosterone system is recommended in patients with arterial hypertension and diabetes mellitus. There are only few prospective data on blood pressure control in patients with diabetes mellitus and impaired renal function with the direct renin inhibitor aliskiren in a daily practice setting available.

Methods: In the non-interventional 3A Registry patients were eligible for inclusion in whom the physician had decided to initiate or modify the anti-hypertensive therapy. This included treatment with the direct renin inhibitor aliskiren or an ACE inhibitor (ACE-I)/angiotensin receptor blocker (ARB) or agents not blocking the renin-angiotensin-system (RAS), alone or on top of an existing drug regimen. Patients were prospectively followed for 1 year. Here we report the results of the prespecified subgroup of patients with diabetes mellitus without (GFR \geq 60 ml/min and with impaired renal function (GFR < 60 ml/min).

Results: Of the 14841 patients recruited by 923 physicians in Germany in 2008 and 2009, 4242 (28%) patients were diabetics. Of these 1334 patients had a GFR < 60 ml/min. The following results with respect to office and 24-hr ambulatory blood pressure after 1 year in the 3 groups were obtained:

	Aliskiren containing regimen	ACE-I/ARB containing regimen	No RAS blockade containing regimen
GFR \geq 60 ml/min			
mean reduction in syst/diast BP (%)	10.2 \pm 12/7.8 \pm 13	8.9 \pm 13/6.6 \pm 14	8.4 \pm 12/6.4 \pm 14
Mean reduction in 24-hr amb. BP (%)	6.8 \pm 10/6.2 \pm 12	5.7 \pm 9/4.0 \pm 12	5.4 \pm 9/6.0 \pm 10
GFR < 60 ml/min			
mean reduction in BP (%)	10.9 \pm 12/7.2 \pm 12	7.4 \pm 12/5.2 \pm 10	8.3 \pm 12/5.5 \pm 12
Mean reduction in 24-amb. BP (%)	6.3 \pm 11/5.2 \pm 12	5.4 \pm 9/4.0 \pm 11	5.9 \pm 9/4.2 \pm 10

Conclusions: In this large real life registry in hypertensive patients with diabetes an aliskiren-containing regimen showed better blood pressure reductions than patients without RAS-blockade, or an ACE-I/ARB-containing regimen, especially in patients with impaired renal function. Therefore treatment with aliskiren might be especially considered in diabetics.

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Is the inverse relationship between blood pressure and mortality in elderly patients with type 2 diabetes caused by heart failure? (ZODIAC-25)

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Background and aims: Several studies have found an inverse association of blood pressure with mortality in elderly patients with type 2 diabetes mellitus (T2DM). Unfortunately, a measure of heart failure, which may be the explanation for the inverse relationship observed, was lacking in those studies. Our aim was to investigate whether adjustment for a measure of heart failure influences the inverse relationship of blood pressure with mortality.

Materials and methods: In 1998, 326 elderly primary care patients with T2DM (>75 years) participated in the ZODIAC study. We found in a recent

study that prognostic properties of MR-proANP are comparable to those of N-terminal pro-B-type natriuretic peptide. Because of skewed distribution, MR-proANP was logarithmically transformed (LnMR-proANP). After a follow-up time of 10 years, updated means for systolic blood pressure, diastolic blood pressure and pulse pressure were calculated. To evaluate the association of the different measures of blood pressure with cardiovascular (CV) and all-cause mortality over time, data were entered as time dependent covariates in a Cox proportional hazard model. We used three different models. In model 1 we adjusted for age, sex, smoking, body mass index, duration of diabetes, serum creatinine, cholesterol-HDL ratio, macrovascular complications, albuminuria and use of lipid lowering and antihypertensive drugs. In models 2 and 3, we additionally adjusted for LnMR-proANP as a continuous variable and MR-proANP categorised into tertiles, respectively.

Results: Median MR-proANP was 125 pmol/L (interquartile range 83-187 pmol/L). During follow-up, 267 (82%) patients died, of which 114 patients (43%) from CV causes. The results of the Cox regression analyses are presented in table 1 (all hazard ratios refer to a pressure increase of 10 mmHg). For CV mortality, inverse relationships were found for systolic blood pressure and pulse pressure. All blood pressure indices were inversely associated with all-cause mortality. Although MR-proANP was independently associated with both CV and all-cause mortality, the inverse relationships between blood pressure indices and mortality did not change after adjustment for MR-proANP.

Conclusion: Although heart failure is highly prevalent in elderly patients with T2DM, the inverse relationship between blood pressure and mortality did not change after adjustment for a measure of heart failure. Based on these results it has become less plausible that heart failure is the only explaining factor for the inverse relationships observed.

Table 1. Hazard ratios for all-cause and cardiovascular mortality

Blood pressure indices	All-cause mortality HR (95%CI)	Cardiovascular mortality HR (95%CI)
Systolic pressure		
Model 1	0.83 (0.76-0.90)	0.83 (0.72-0.94)
Model 2	0.83 (0.75-0.90)	0.82 (0.72-0.93)
Model 3	0.83 (0.75-0.90)	0.82 (0.71-0.93)
Diastolic pressure		
Model 1	0.82 (0.69-0.97)	0.88 (0.68-1.13)
Model 2	0.79 (0.67-0.93)	0.87 (0.67-1.12)
Model 3	0.80 (0.68-0.95)	0.86 (0.67-1.12)
Pulse pressure		
Model 1	0.81 (0.72-0.90)	0.78 (0.66-0.91)
Model 2	0.81 (0.73-0.90)	0.78 (0.66-0.92)
Model 3	0.80 (0.72-0.90)	0.78 (0.66-0.91)

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Increased protein intake is associated with uncontrolled blood pressure evaluated by 24-h ambulatory blood pressure monitoring in type 2 diabetes

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Background and aims: Dietary intervention is an important component of hypertension management. However, evidence for dietary recommendation in patients with type 2 diabetes came from studies in non-diabetic individuals and evaluated only office blood pressure (BP) levels. Ambulatory blood pressure monitoring (ABPM) allows evaluation of BP homeostasis abnormalities not detected in office setting which have been associated with diabetic chronic complications. The aim of the present study was to analyze possible associations of usual diet with BP homeostasis in patients with type 2 diabetes. **Material and methods:** In this cross-sectional study 124 outpatients with type 2 diabetes (mean age: 62.4 years; mean diabetes duration: 12 years; 54% women; white ethnicity 73.4%; 81.5% hypertensive on office measurements) and without dietary counseling during the previous six months had their daily intake assessed by 3-day weighed-diet records (Nutribase 2007-updated). Reliability of records was confirmed by 24-h urinary nitrogen output. BP was assessed by ABPM (Spacelabs 90207). Patients were divided into two groups

according their daytime BP records on ABPM: uncontrolled-BP (systolic BP \geq 135 mm Hg and/or diastolic BP \geq 85 mm Hg) and controlled-BP (systolic BP $<$ 135 mm Hg and diastolic BP $<$ 85 mm Hg).

Results: Patients with uncontrolled-BP ($n=81$) had higher office systolic BP [147.4 \pm 25.2 vs. 134.2 \pm 19.5 mm Hg, $P=0.004$] and urinary albumin excretion [UAE, median (interquartile range); 7.7(37) vs. 4.9(95) mg/24-h, $P=0.04$] as compared to those with controlled-BP ($n=43$). Concerning dietary intake, patients with uncontrolled-BP had higher intake of protein [% energy (%en)] than patients with controlled-BP (20.0 \pm 3.7 vs. 18.2 \pm 3.6 %en, $P=0.02$). Lower carbohydrate intake (46.5 \pm 6.7 vs. 49.0 \pm 6.9 %en, $P=0.07$), higher HbA1C (8.3 \pm 2.0 vs. 7.6 \pm 1.3%, $P=0.06$), and higher BMI (29.9 \pm 4.9 vs. 28.5 \pm 4.3 kg/m², $P=0.13$) occurred in those with uncontrolled-BP as compared with controlled BP group, but statistical significance was not reached. Protein intake was associated with a 13% increased chance for having uncontrolled-BP in univariate analysis (OR 1.13; 95%CI 1.019–1.260; $P=0.02$). This association remained in a multivariate logistic regression model adjusted for BMI, HbA1C, and UAE (OR 1.13; 95%CI 1.008–1.272; $P=0.03$). Considering protein sources, meat intake [median (interquartile range) = 2.6(1.6) vs. 2.0(1.1) g/kg; $P=0.04$] was increased in patients with uncontrolled-BP, with a linear increase in the proportion of patients with uncontrolled-BP throughout quintiles of protein consumption (P for trend=0.04). Meat intake also had a linear association with 24-h systolic BP in a multivariate linear model, adjusted for BMI, HbA1c, and UAE (adjusted R² 25%; $P=0.02$). No association of ABPM measurements with carbohydrate intake was demonstrated.

Conclusion: Increased protein intake, especially from meat sources, was associated with an increased chance of having high ABPM values in patients with type 2 diabetes. Further studies are needed to clarify the role of reducing meat intake in hypertension management in patients with type 2 diabetes.

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Tonometric ambulatory blood pressure measurements are reliable and improve hypertension diagnostics in patients with type 1 diabetes

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Background and aims: Blood pressure control is paramount in clinical management of type 1 diabetes (T1DM). A novel approach to blood pressure measurements is a tonometric technique, where a device (Bpro) captures radial pulse wave reflection and calculates brachial arterial blood pressure (BP). Tonometric BP measurements allow for more frequent and potentially more reliable BP measurements than cuff-based ambulatory (AMBP) measurements due to less discomfort and less anticipation BP rise in connection with BP measurements. The diagnosis of hypertension is often based on office blood pressure (OBP) rather than AMBP measurements. We suggest that OBP is suboptimal in hypertension diagnostics. In this study we investigate if a tonometric device is applicable and reliable in patients with T1DM, and whether OBP is reliable in hypertension diagnostics in T1DM.

Materials and methods: In a cohort of 24 Caucasian patients with diabetes we compared tonometric BP measurements with Bpro to a conventional, cuff-based device (Takeda TM2421). Patients were seen twice within 2 weeks. At visit 1, 15 minutes rest was followed by three Takeda measurements and 2 minutes continuous Bpro BP measurements. At both visits AMBP measurements were recorded using the Bpro. In a different cohort we included 326 Caucasian T1DM patients, 178 men (55%), aged (mean \pm SD) 54 \pm 13 years. Normoalbuminuria was present in 160 patients, whereas 166 patients had micro- or macroalbuminuria (>30 mg/24h), with creatinin levels of (mean \pm SD) 73 \pm 14 and 126 \pm 51 μ mol/l, respectively. All had been regularly attending clinical controls and were randomly selected for AMBP with a Bpro. Mean OBP was calculated from cuff-based BP measurements at three separate office visits $<$ 1 year prior to AMBP. AMBP and OBP values \geq 130/80 mmHg were classified as hypertension (acc. to ADA guidelines). Patients with normal OBP, but elevated AMBP were classified as masked hypertensives, and patients with elevated OBP, but normal AMBP were classified as white coat hypertensives.

Results: Validation: At visit 1, Takeda BP values (mean \pm SD) were 136 \pm 19/72 \pm 8 mmHg vs. Bpro values of 138 \pm 19/78 \pm 8 mmHg. The visit 1 Bpro AMBP was 132 \pm 20/76 \pm 9 mmHg vs. 131 \pm 13/75 \pm 9 mmHg at visit 2. Correlations between the Takeda and Bpro systolic and diastolic BP were $r=0.86$ and 0.65 , respectively ($p<0.001$). The mean differences (\pm SD) between devices were 1.9 \pm 10 and 5.5 \pm 6.6 mmHg for systolic and diastolic BP, respectively. Comparison of Bpro systolic and diastolic AMBP at visit 1 and 2 showed no difference in mean BP ($p=0.81$ and $p=0.76$, respectively). Neither mean day BP, mean

night BP nor mean dipping were statistically different ($p>0.40$) between the two visits. Evaluation: OBP values were (mean \pm SD) 136 \pm 14/76 \pm 8 mmHg vs. AMBP 129 \pm 15/76 \pm 10 mmHg. Fifty-nine patients (18%) were normotensive and 153 (47%) had sustained hypertension with both AMBP and OBP. White coat hypertension was present in 82 (25%) and masked hypertension in 32 (10%) patients. Thus, 65% were diagnosed correctly by OBP, assuming AMBP reveals the correct BP. Overall, 185 patients (57%) did not reach target BP of $<$ 130/80 mmHg, as they had either sustained (47%), or masked (10%) hypertension.

Conclusion: In patients with diabetes tonometric BP measurements are comparable to cuff based measurements, and tonometric AMBP measurements are reproducible. In T1DM, AMBP is necessary to diagnose hypertension in 35% of patients. Despite regular follow-up, the majority of patients did not reach target BP.

Clinical Trial Registration Number: NCT01171248

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Longer term effect of pregnancy induced hypertension on chronic kidney disease and hypertension: a case-control study

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Background and aims: Pregnancy Induced hypertension (PIH) with its proteinuric (PE) and nonproteinuric (GH) subtypes are known to be a major cause of maternal and fetal morbidity and mortality. In addition, PIH is claimed to create greater risk of hypertension (HTN) and chronic kidney diseases (CKD) at later life of the women. The nature and extent of HTN and CKD along with the interrelation of the two disorders are still not fully settled. The present study was designed to explore what proportion of women with history of PIH develop HTN and CKD in later stages after delivery and, at the same time, to study the interrelation of HTN and CKD with their socio-demographic, anthropometric and biochemical risk factors.

Materials and methods: Under an observational case-control design 133 women with previous history of PIH [the PIH group; Age (yrs), Median (range) 31 (25–45), and BMI (kg/m², (Mean \pm SD) (25.2 \pm 2.1)] were compared with 113 women without history of PIH (Non-PIH group), Age (yrs) Median (range) 34 (25–45), and BMI [(kg/m², Mean \pm SD) (25.8 \pm 2.9)] for the development of HTN (SBP $>$ 130 mmHg; DBP $>$ 90 mmHg; or MBP $>$ 105 mmHg) and CKD (as measured by total urinary protein and elevated UPCR). Clinical and anthropometric parameters were measured by standard techniques, lipids were measured by enzymatic-colorimetric method, urinary total protein by pyrogallol red method, urinary protein by strip method and urinary creatinine was measured by alkaline picrate method.

Results: Out of the 133 subjects in the PIH group 43(32.3%) developed HTN and 41 (30.8%) developed CKD. In the Non-PIH group 17(15%) developed HTN and 16 (14.2%) developed CKD. SBP, DBP and MBP, all were significantly higher in the PIH compared to the Non-PIH group. PIH subjects had 4 times higher chance of developing HTN (Odds Ratio 4.2). In parallel to the findings on HTN the PIH group showed a significantly higher proportion of CKD [Proteinuric 10 (7.5%)]. Both urinary total protein and urinary protein-creatinine ratio were significantly higher in the PIH group as compared to the Non-PIH group. The PIH subjects had almost 3 times chance of developing CKD compared to the Non-PIH group. A significant correlation of MBP was found with UPCR, Tchol and serum uric acid. On the other hand UPCR was also correlated with SBP. On logistic regression MBP had a positive association with UPCR and Uric Acid.

Conclusion: About one-third of women with a previous history of PIH develop HTN in later life and the proportion is two times higher and the risk is 4 times more than that in the Non-PIH counterparts. About 8% of women with previous history of PIH develop chronic kidney disease in later life and the proportion is 5 times higher to develop and they are almost 3 times more vulnerable than that in the non-PIH group.

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Sodium glucose co-transporter-2 (SGLT2) inhibitor, PF04971729, reduces blood pressure and body weight in spontaneously hypertensive rats (SHR)

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Background and aims: Studies suggest that SGLT2 inhibition results in a favorable effect on blood pressure (BP) in type 2 diabetics with mild hypertension and obesity. Since SGLT2 inhibition not only reduces hyperglycemia and body weight (BW), but also produces diuresis via an increase in urinary glucose excretion (UGE), it would be important to determine if SGLT2 inhibition reduces BP in a hypertensive model without the hyperglycemia and obesity associated with type 2 diabetes. Thus, the goal of this study was to evaluate the BP lowering effects of a new, highly potent and selective SGLT2 inhibitor, PF04971729, in the lean, euglycemic SHR model of hypertension.

Materials and methods: Age-matched (13 week old), male SHR were previously instrumented using aseptic techniques to measure aortic BP via telemetry. After recovery from surgery and acclimation to telemetry housing, the rats were allocated into two study groups (control and PF04971729-treated, $n = 8$ /group) based on BW and systolic BP. PF04971729-treated SHR were paired to match the amount of chow consumed per day by the control group. PF04971729 was delivered in an admixture (0.5 mg PF04971729/g chow) to produce a near-maximal increase in UGE. Blood pressure measurements over 24 h were obtained at baseline (Day 0) and after 27 days of treatment. All rats were then placed in metabolic cages for 24-h urine collection followed by blood sample collection.

Results: All data are Mean \pm SEM. Statistical significance ($p < 0.05$) vs baseline or control group was calculated using ANOVA. Baseline BP values were similar between the control and PF04971729-treated hypertensive SHR (see table). At the end of treatment, PF04971729 increased ($p < 0.05$) UGE to 3636 ± 111 mg/24 h compared to the control group (7 ± 3 mg/24 h); however, plasma glucose levels (140 ± 11 mg/dl) were not different compared to the control group (147 ± 21 mg/dl) in the euglycemic SHR. PF04971729 (vs control) increased ($p < 0.05$) urine volume to 42 ± 1 vs 14 ± 1 mL/24 h, increased ($p < 0.05$) hematocrit to 53 ± 1 vs 49 ± 1 %, and increased ($p < 0.05$) plasma renin activity to 21 ± 3 vs 7 ± 1 ng/ml/h, consistent with a diuretic effect. On day 27, PF04971729 significantly lowered systolic BP and mean BP compared to baseline and control group; the decrease in diastolic BP in the PF04971729-treated rats did not reach statistical significance (see table). Heart rate was slightly lower (-9 ± 4 beats/min) in the control group but significantly ($p < 0.05$) lower (-49 ± 7 beats/min) in the PF04971729-treated SHR. In contrast to the control group, PF04971729 by day 27 caused a 12 ± 1 % loss ($p < 0.05$) in BW from baseline (307 ± 4 g).

Conclusion: This study for the first time demonstrates a nonclinical BP lowering with SGLT2 inhibition in a lean, euglycemic SHR model of hypertension. The BP lowering was associated with significant diuretic effects that may be exaggerated by the loss of BW in the pair-fed SHR. The magnitude of BP lowering over 24 h with PF04971729 in hypertensive, type 2 diabetic patients is under clinical evaluation.

Effects of PF04971729 on 24h BP measurements in SHR

	Control (n=8)		PF04971729 (n=8)	
	Day 0	Day 27	Day 0	Day 27
Systolic BP (mmHg)	166 \pm 2	170 \pm 5	167 \pm 2	151 \pm 5*†
Diastolic BP (mmHg)	111 \pm 2	115 \pm 5	110 \pm 2	101 \pm 5
Mean BP (mmHg)	138 \pm 2	143 \pm 5	138 \pm 2	125 \pm 5*†

Mean \pm SEM; * $p < 0.05$ vs baseline; † $p < 0.05$ vs control (ANOVA)

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Blood pressure lowering effects of the direct renin inhibitor aliskiren in patients with diabetes: a pooled analysis of 16 randomised trials

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Background and aims: Diabetes mellitus and hypertension is typically associated with activation of the renin angiotensin aldosterone system (RAAS) leading to poorer clinical outcomes compared to a general hypertensive pop-

ulation. The aim of this pooled analysis was to evaluate the blood pressure (BP) lowering efficacy of aliskiren monotherapy in hypertensive patients with diabetes.

Materials and methods: Data was pooled from pooled from 16-randomized, double-blind clinical trials to evaluate the blood pressure (BP) lowering efficacy of aliskiren monotherapy (150-300 mg) over 8-12 wks in 879 hypertensive patients with diabetes.

Results: The mean baseline values for the total population ($n = 10875$) and the patients with diabetes ($n = 1672$) were as follows; systolic BP $154.3 \pm 12.0/156.3 \pm 11.8$ mmHg; diastolic BP $97.4 \pm 6.5/96.3 \pm 7.2$ mmHg; $56.7 \pm 12.1/60.5 \pm 10.9$ yrs (57/60 % male), BMI 29.4 ± 5.9 ($37\% \geq 30$ kg/m²)/ 31.1 ± 6.0 ($50\% \geq 30$ kg/m²); eGFR, $88.8 \pm 20.8/88.1 \pm 23.3$ ml/min and fasting glucose $5.9 \pm 1.5/7.8 \pm 2.5$ mmol/L. The patients with diabetes in response to aliskiren monotherapy had significant dose-related reductions in systolic and diastolic BP which were similar to the overall patients (Table). However the systolic BP lowering effect of aliskiren was similar between the diabetic patients with fasting glucose levels ≥ 7 mmol/L (-15.2 ± 13.1 mmHg; $n = 420$) and those below 7 mmol/L (-14.3 ± 14.0 mmHg; $n = 332$). Baseline plasma renin activity and aldosterone levels for patients with diabetes (0.53 ng/ml/h; 169 pmol/L) were similar to those of the overall hypertensive patients (0.57 ng/ml/h; 171 pmol/L).

Conclusions: The BP lowering results in hypertensive patients with diabetes demonstrated that despite greater challenge for these patients to reach a target BP goal their responsiveness to direct renin inhibition with aliskiren monotherapy was similar to the overall hypertensive patients in clinical trials. Further, the antihypertensive response to aliskiren in patients with diabetes was not influenced by baseline glycemic status or activation of the renin angiotensin system. The data also suggest that the effectiveness of aliskiren is not associated with elevations in plasma renin in patients with diabetes.

Mean change in blood pressure

Treatment	All patients (n)	Patients with diabetes (n)
Placebo	SBP -6.1 \pm 12.9 (1728)	-6.8 \pm 14.2 (151)
	DBP -5.45 \pm 8.2	-5.7 \pm 8.1
Aliskiren 150 mg	SBP -13.1 \pm 13.1 (2613)	-12.7 \pm 13.7 (309)
	DBP -9.0 \pm 8.2	-7.8 \pm 8.7
Aliskiren 300 mg	SBP -16.6 \pm 13.7 (3310)	-15.2 \pm 13.1 (575)
	DBP -10.65 \pm 8.4	-11.6 \pm 8.3

Data shown are mean \pm SD changes from baseline in SBP and DBP (mmHg). SBP, systolic blood pressure; DBP, diastolic blood pressure.

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Effect of bariatric surgery on the mechanisms involved in obesity-related hypertension: the renin-angiotensin-aldosterone and sympathetic nervous systems and endothelial dysfunction

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Background: The activation of the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system (SNS), endothelial dysfunction and hyperleptinemia has been implicated in the pathogenesis of obesity-related hypertension (HT).

Aim: We evaluated the effect of significant and sustained weight loss obtained by bariatric surgery (BS) on blood pressure (BP) and their main mechanisms of regulation.

Methods: Patients with severe obesity and documented HT who underwent laparoscopic BS (bypass gastric or sleeve gastrectomy) were studied. Patients were evaluated before BS and 4 and 12 months post-operatively. Antihypertensive treatment was withdrawn one week before each evaluation. Anthropometric data were collected and BP (24-h ambulatory BP measurement) assessment and blood samples were analysed for RASS [plasma renin activity (PRA), aldosterone, angiotensin II and angiotensin converting enzyme], SNS (metanephrine, normetanephrine and noradrenaline), insulin and leptin values. Endothelial function was determined by endothelial-dependent flow-mediated vasodilatation on the brachial artery and the ratio of the intra-

abdominal visceral fat area to the subcutaneous fat area (V/S ratio) was calculated using an abdominal scanner.

Results: Twenty-five patients were studied; 16 females, aged 51 (37–64) years, with HT evolution of 6 (1–20) years and excess body weight of 55 (36–76) kg. Twelve months after BS: the BMI decreased from 46 to 32 Kg/m²; excess body weight loss was 69 (16) %; 64% patients had complete resolution of HT while 32 % patients had improvement; 24-h (systolic -17 (14)/diastolic -6 (11) mmHg), daytime and night-time BP values decreased significantly. The PRA (0.26 to 0.19 ng/mL*h), aldosterone (5.5 to 3.2 ng/dl), nor-adrenaline (117 to 91 pg/mL), insulin (33 to 10 mU/L) leptin (67 to 20 ng/ml) and V/S ratio (0.39 to 0.24 cm²) also significantly decreased ($p < 0.05$). We observed a direct relationship between decrease in 24 h and night-time systolic BP and reduction of nor-adrenaline ($r^2 = 0.22$, $p = 0.03$ and $r^2 = 0.39$, $p = 0.003$, respectively). No significant differences were observed in endothelial-dependent flow-mediated vasodilatation after BS.

Conclusion: Weight loss after BS is associated with a reduction in BP and RAAS and SNS activity in hypertensive obese patients.

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PS 099 Retinopathy: experimental

1108

Prorenin stimulates retinal endothelial cell proliferation: implications for diabetic retinopathy

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Background and aims: The renin-angiotensin system (RAS) contributes to the development of diabetic retinopathy (DR), one of the most feared complications of diabetes. Pre-clinical studies and clinical trials such as Diabetic REtinopathy Candesartan Trial (DIRECT) have reported that type 1 angiotensin receptor blockade (ARB) attenuates vasculopathy in DR. Despite these findings, ARB does not provide full protection in DR, suggesting the involvement of other mechanisms. Prorenin, the inactive precursor of renin, which initiates the RAS, is present at increased concentration in plasma and vitreous fluid of patients with proliferative DR. However, whether prorenin itself contributes to retinal endothelial cell proliferation is unknown. This study aims to determine whether prorenin influences retinal endothelial cell proliferation and whether this involves the (pro)renin receptor [(P)RR].

Materials and methods: The proliferation of primary bovine retinal endothelial cells (BREC) was measured following treatment with a range of concentrations of prorenin (0.02 to 20nM). To determine if prorenin's influence on BREC proliferation involved the type 1 angiotensin receptor or renin, BREC were co-incubated with prorenin (2nM) plus candesartan (10μM) or the renin inhibitor aliskiren (10μM). Real-time PCR was performed to measure the expression of the angiogenic factors; vascular endothelial growth factor (VEGF), VEGF Receptor 2 (VEGFR2) and tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (Tie2), and the (P)RR, after a time course of 2nM prorenin stimulation.

Results: Prorenin promoted BREC proliferation by $43.74 \pm 11.36\%$ ($p < 0.05$), whereas candesartan alone had no effect, and aliskiren alone reduced proliferation by $54.15 \pm 0.29\%$ ($p < 0.001$), compared to untreated control. Candesartan pretreatment had no effect on the stimulation of BREC proliferation by prorenin. Although aliskiren pretreatment reduced BREC proliferation, subsequent addition of prorenin to aliskiren-treated BREC increased proliferation above that seen with aliskiren alone. Prorenin increased VEGF, VEGFR2, Tie2 and (P)RR mRNA levels (1.1 to 1.3 fold, $p < 0.05$) in a time dependent manner (between 2 and 8 hours).

Conclusion: This study demonstrated that prorenin, independently of type 1 angiotensin receptor activity or renin activity, stimulated pathological events associated with the development of DR such as endothelial cell proliferation and the expression of angiogenic factors. The finding that (P)RR mRNA levels are altered, suggests that prorenin may mediate its effects on retinal vascular proliferation via the (P)RR.

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1109

Anti-apoptotic effect of angiotensin-II receptor blockade in human retinal pericytes cultured in diabetic-like conditions

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Background and aims: Diabetic retinopathy is characterized by damage to the retinal microvasculature. Capillary degeneration, which includes thickening of the basement membrane and pericyte drop-out, begins early and, as it becomes more extensive, contributes to large areas of capillary non-perfusion characteristic of advanced diabetic retinopathy. Angiotensin II (Ang II) regulates several pathological events, including cell proliferation/hypertrophy, proinflammatory responses and extracellular matrix degradation. Elevated Ang II and/or increased sensitivity to Ang II have been etiologically associated with major vascular diseases. Most of the studies on the effects of Ang II on vascular cells, however, have been done on smooth muscle cells while an emerging role for Ang II in the modulation of retinal vascular cell function in diabetes has been recently postulated. Clinical studies have suggested that inhibitors of the renin-angiotensin system (RAS) may slow the progression of mild to moderate diabetic retinopathy. The objective of this study was to verify if the Ang II receptor blocker candesartan can act as an anti-apoptotic and protective factor for human retinal pericytes (HRP) in diabetic-like conditions, counteracting the negative effects of high glucose.

Materials and methods: Pericytes were kept alternatively in high (28 mM, HG) or normal (5.6 mM, NG) glucose at 48h intervals for 8 days (intermittent HG, intHG), with or without 10, 2, 1 or 0.2 μ M candesartan. Control cells were cultured in stable NG or HG. Apoptosis was determined by ELISA, as DNA fragmentation, proliferation by cell counts and BrdU incorporation. Senescence-associated lysosomal activity by β -galactosidase staining was performed as a biomarker for cellular aging and observed at the light microscopy. **Results:** Intermittent but not stable HG increased apoptosis in HRP (+52.5%, $p < 0.05$ vs NG), consistently with our previous findings showing that exposure to intermittent but not steadily high glucose increases apoptosis in human pericytes. Candesartan 1 and 0.2 μ M, added to intermittent HG, was able to normalize apoptosis (1 μ M candesartan: -30.3%, 0.2 μ M candesartan: -42.8% vs intHG, $p < 0.05$), while higher concentrations had no significant effects. Addition of candesartan seemed to have no influence on proliferation, but it was able to reduce intermittent high glucose-activated senescence.

Conclusion: Candesartan has a significant anti-apoptotic and anti-senescence effect on human retinal pericytes grown in diabetic-like conditions. Further studies are necessary to better understand the mechanisms through which it works, in particular the signalling pathways involved and/or influenced by the RAS.

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FAD286, an aldosterone synthase inhibitor, reduces retinal neovascularisation

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Background and aims: Blockade of the type 1 angiotensin receptor (ARB) is a potential treatment for microvascular disease in diabetic retinopathy. We previously identified that inhibition of the mineralocorticoid receptor (MR) also reduces vasculopathy in experimental diabetic retinopathy. However, it remains to be determined whether aldosterone or corticosterone, which both bind to the MR, are responsible for MR stimulation on retinal neovascularization. The aim of this study was to determine if inhibition of aldosterone with the aldosterone synthase inhibitor, FAD286, reduces retinal neovascularization, and whether it is as effective as ARB. Additionally, we evaluated the cellular source of aldosterone synthase in retina.

Materials and methods: Oxygen-induced retinopathy (OIR) was induced in Sprague Dawley rats (80% O₂, 22hrs/day) from postnatal days (P) 0 to P11, followed by 7 days in room air. Control rats were always in room air. FAD286 (30mg/kg/day), the ARB valsartan (10mg/kg/day) or a combination of FAD286+valsartan was administered between P12 to P18. Primary cultures of rat retinal microglia, rat glia, rat ganglion cells, bovine retinal endothelial cells and bovine retinal pericytes were studied.

Results: In OIR, FAD286 reduced pre-retinal neovascularization and neovascular tufts by approximately 89% and 67% respectively compared to untreated OIR, and to a similar extent as valsartan and combination treatment. In OIR, retinal mRNA and protein levels of the potent angiogenic factor, vascular endothelial growth factor, were increased by 1.7 and 4.74 fold respectively compared to control ($P < 0.05$), and reduced to a similar extent with FAD286, valsartan and combination treatment. In OIR, FAD286 reduced the retinal mRNA levels of the inflammatory mediators: tumor necrosis factor- α , intercellular adhesion molecule-1, vascular adhesion molecule-1 and monocyte chemoattractant molecule-1 to a similar extent as valsartan and combination treatment ($P < 0.05$ to OIR). In retina, FAD286 reduced both aldosterone synthase mRNA and aldosterone levels, whereas hydrocorticosterone levels were unchanged. In vitro, microglia and ganglion cells were identified as the major sources of aldosterone synthase mRNA and all cell types expressed MR mRNA. In OIR, the density of microglia (Iba1 immunolabelling) was increased in OIR (2.54 ± 0.33) compared to controls (0.21 ± 0.02), and reduced to a similar extent with FAD286 (1.24 ± 0.18 , $P < 0.05$ to OIR) and other treatments.

Conclusion: The finding that inhibition of aldosterone production per se, is equally effective as ARB in ameliorating retinal neovascularization, suggests that aldosterone may be needed to be considered when evaluating treatments for diabetic retinopathy that involve blockade of the renin-angiotensin system. The localization of aldosterone synthase to retinal microglia and ganglion cells suggests that these cell types participate in the neovascular response induced by aldosterone.

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Effects of atorvastatin and insulin in the retina of an animal model of type 2 diabetes with hyperlipidaemia

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Background and aims: Insulin resistance, a key feature of obesity, the metabolic syndrome and T2D, results in a variety of metabolic and vascular abnormalities. Endothelial dysfunction is intricately related to insulin resistance through the parallel stimulatory effects of insulin on glucose disposal in metabolic tissues. Perturbations characteristic of insulin resistance, including dyslipidaemia, inflammation and oxidative stress, may together contribute to disrupt the structural or functional integrity of the retina endothelium. The purpose of this study is to investigate the retinal cells damage in diabetic and high-fed animal models and elucidate the potential mechanisms underlying the benefits of therapy with atorvastatin or insulin.

Materials and methods: The proteasome activities were assessed using fluorogenic substrates in non-diabetic animals (Wistar rats), untreated Goto-Kakizaki rats (diabetic rats), high-fat fed GK rats (fed with atherogenic diet only for 4 months, treated with insulin or atorvastatin for the last month). Production of oxygen reactive species was determined by immunofluorescence in frozen retina sections using dihydroethidium, in the different groups of animals. Protein levels of ubiquitin conjugates, free ubiquitin, E1, I κ B and NF- κ B were determined by Western Blotting. Cell death was determined by evaluation of the levels of Bax, Bcl-2 and caspase-3 by immunofluorescence and immunoblot analysis. Data were expressed as mean \pm SEM. ANOVA followed by Bonferroni's post hoc test. $P < 0.05$ was considered statistically significant.

Results: Diabetes and atherogenic diet significantly induced an increase in the production of superoxide anion in retinas (from 100% to $158 \pm 12.5\%$ and $195 \pm 13.6\%$, respectively). Our data indicates also a significant decrease in chymotrypsin-like (to $39.9 \pm 0.02\%$) and post-glutamyl peptide hydrolytic-like (to $75.1 \pm 0.01\%$) activities and unchanged tryptic-like activity when the diabetic animals were fed with atherogenic diet. Statins significantly reverted the levels of oxidative stress and recovered the proteasome activities to similar levels of control rat retinas. The atherogenic fat diet induced a significant decrease in the high molecular weight ubiquitin conjugates (to $58.3 \pm 5.9\%$) paralleled with an increase in free ubiquitin. This may occur as a result of degradation of E1-ubiquitin activating enzyme by calpains, being a limiting step in the formation of the ubiquitin protein conjugates. Diabetes increases the ratio of Bax/Bcl-2 proteins. We also observed that atherogenic diet induced a hyperphosphorylation of Bcl-2 and its inactivation, leading to caspase-3 cleavage and cell death by apoptosis. Bcl-2 inactivation led to degradation of I κ B, allowing NF- κ B to enter the nucleus and induce gene expression.

Conclusion: Increased oxidative stress in diabetic retinas from animals with obesity leads to inactivation of the 20S proteasome activity and increased cell death by apoptosis. Atorvastatin monotherapy restores the retina cell function and significantly improves local oxidative stress in high-fat fed GK rats. Improved retinal cells function due to statins was at least partially to the ubiquitin proteasome pathway restoration, and represents a pharmacological approach to prevent some of the complications associated with diabetic retinopathy.

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S-nitrosoglutathione ameliorates the early marker of diabetic retinopathy through oxidative status balance

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Background and aims: The chemistry associated with the production of nitric oxide (NO) in biological systems provides arguments from which the diverse functions of NO may be interpreted. NO is a readily diffusible, short-lived molecule produced by the action of NO synthase (NOS) on L-arginine. In the nervous system, NO is a neurotransmitter and can act both intracellularly as a second messenger and extracellularly as a conveyor of information between cells and as a neuromodulator. On the other hand, NO signaling in endothelial cells regulates vascular permeability and blood flow, and the uptake and response to therapeutic compounds. S-nitrosoglutathione (GSNO), a possible carrier of NO, may extend the *in vivo* actions of locally produced NO. GSNO has been shown to be several-fold more potent than reduced

glutathione (GSH) against oxidative stress. N-nitrosation of amines to form N-nitrosamines (RNNOs) has been studied mainly in the context of carcinogenesis with no information in diabetic retinal disease. The aim of this study was to investigate the efficacy of GSNO topically (eye drop) in preventing the early retinal change in an *in vivo* and *in vitro* study.

Materials and methods: 4-week-old spontaneously hypertensive rats (SHR) were rendered diabetic by intravenous injection of streptozotocin (50 mg/kg), the control rats received only citrate buffer. Diabetic SHR rats were randomized to receive no treatment or treatment with GSNO topically twice a day (600 nM) for 20 days. *In vitro* studies were performed with human retinal pigment epithelial cell (ARPE-19) cultured in normal glucose (NG; 5mM), high glucose (HG; 30mM) and high glucose (HG; 30mM) plus GSNO treatment with 1, 10 and 100nM, 1 and 10μM for 24 hours after MTT cytotoxicity assay. Intracellular reactive oxygen species (ROS) production was assessed by 2',7'-dichlorofluorescein diacetate (H₂DCFDA). The results were compared by Analysis of Variance (ANOVA) followed by Fisher's protected least significant difference test.

Results: As expected, body weight was lower and glycaemia was greater in diabetic SHR's than in non-diabetic rats ($p<0.0001$); the systolic blood pressures were equal in all studied groups. The early molecular marker of diabetic retinopathy (DR), evaluated through glial reaction by expression of glial fibrillary acidic protein (GFAP), was significant increased in diabetic SHR compared with control SHR rats ($p=0.005$). The eye drop treatment in diabetic SHR prevented this increment. In ARPE-19 cells, there was an increasing in ROS production under HG compared with NG ($p=0.02$) and the treatment with GSNO (1, 10 and 100nM) reestablished to normal levels.

Conclusion: Topical treatment with GSNO eye drop prevented the increase of GFAP in diabetic rats and *in vitro* abolished the ROS production in ARPE-19 under HG. The molecular mechanisms of GSNO actions in the diabetic retinas are being assessed in *in vitro* studies.

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1113

HM-VN118, a herbal medicine, inhibits the development of diabetic retinopathy and retinal AGEs accumulation in spontaneous diabetic Torii rats

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Background and aims: Diabetes alters the structure and function of most cell types in the retina, including the vasculature and neural network. The loss of retinal capillary cell and neuronal cell is a hallmark of diabetic retinal changes. Advanced glycation end products (AGEs) are believed to contribute to retinal cell loss in diabetic retinopathy. The aim of this study was to investigate the potential preventive effect of HM-VN118, a herbal medicine, on diabetic retinopathy in spontaneous diabetic Torii (SDT) rat, and animal model of type II diabetes.

Materials and methods: Twenty five week-old male SDT rats were treated with HM-VN118 (100 and 250 mg/kg body weight) once a day orally for 17 weeks. AGEs accumulation, pericyte loss, blood retinal barrier breakage and ganglion cell injury were investigated in retinas from SDT rats.

Results: Vehicle-treated SDT rats exhibited hyperglycaemia and diabetic retinopathy. However, the treatment of HM-VN118 decreased significantly AGEs levels in retinal tissues ($p<0.01$ vs vehicle). In fluorescein angiography, the changes of retinal vasculature, such as fluorescein leakage and vessel narrowing were significantly reduced in SDT rats treated with HM-VN118. In addition, the retinal vascular disorders such as pericyte loss and acellular capillary formation were highly observed in SDT rats, but in SDT rats treated with HM-VN118, these changes were observed rarely. Similarly, diabetes-induced microvascular and neuronal cell apoptosis was also reduced in HM-VN118-treated SDT rats ($p<0.05$ vs vehicle).

Conclusion: These results suggest that HM-VN118 is useful in inhibiting the AGEs accumulation in diabetic retinas and may have therapeutic potential in the treatment of diabetic retinopathy.

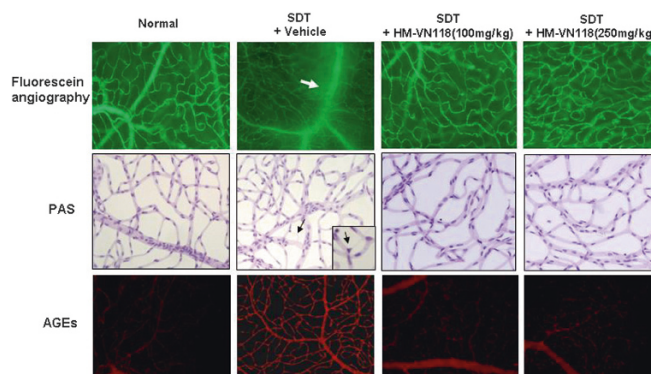


Figure 1. Effects of HM-VN118 on retinal vascular hyperpermeability (upper panels), microvascular cell loss (middle panels) and AGEs accumulation (low panels) in SDT rats. In fluorescein angiography, white arrow indicates the vascular leakage. In PAS staining, black arrowhead and arrow indicate acellular capillary and migrating pericyte (magnified inset), respectively. Retinal AGEs accumulation (red) was observed in SDT rats.

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1114

Green tea polyphenols protect retinal cells in high glucose condition

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Background and aims: In our previous studies, we have demonstrated that green tea polyphenols (GTPs) attenuate the early changes in the retina from experimental diabetic rats, such as glial fibrillary acidic protein (GFAP), tight junction occludin and nitrotyrosine expression, blood retinal barrier breakdown and retinal function evaluated by electroretinogram. The ARPE-19 cell line is a human retinal pigment epithelium (RPE) and has been used in studies to evaluate not only the outer blood retinal barrier (BRB) but also the retinal neuroprotection since these cells exhibit glutamate transporters. Glial cells (especially Muller cell) have a central role in the homeostasis in the retina including maintenance of BRB and the neural retinal elements from the excessive stimulation of neuronal NMDA-sensitive glutamate receptors. Thus, the mechanisms to remove glutamate from the extracellular space are required for efficient maintenance of a healthy retina. The aims of this study were to investigate the possible mechanisms involved into the protective effects of GTPs on Muller and retinal pigment epithelium cells (ARPE-19) exposed to high glucose.

Materials and methods: For these studies, we used ARPE-19 and Muller cells. The cells were exposed to normal glucose (NG; 5mM), high glucose (HG; 30mM) in presence or absence of GTPs treatment and high glucose (30mM) with epigallocatechin gallate (EGCG) for 24 hours. In ARPE-19, the cytotoxicity was determined by MTT assay for treatments with GTPs and EGCG. 100 μg/ml was selected for GTPs and 50, 25 and 10μM for EGCG for the experiments. The total intracellular reactive species (ROS) was determined by 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) method. The expression on cell lysates of EAAC1 (glutamate transporter) was determined by western blot. In Muller cells, we analyzed the expression of GFAP by immunofluorescence and glutamate transporter (Glast) and glutamate receptor (NMDAR1) by Western blot. The results were compared by Analysis of Variance (ANOVA) followed by Fisher's protected least significant difference test.

Results: In ARPE-19 cells, cultured under HG for 24 hours, there was an increasing in ROS production compared with NG ($p<0.0001$). The presence of GTPs prevented this abnormality ($p=0.001$); similar findings were observed in presence of EGCG under HG condition ($p=0.001$). The protein expression of EAAC1 was increased in HG and reversed with GTPs ($p<0.05$). In Muller cells, the levels of GFAP evaluated by immunofluorescence were increased in HG compared with NG and the treatment with GTPs abolished this effect. Glutamate transporter expression and its receptor decreased in HG ($p<0.05$) and the treatment with GTPs reestablished ($p<0.05$).

Conclusion: GTPs prevented the ROS and EAAC1 increments in ARPE-19 cells. In the Muller cells, there was a significant reestablishment of glutamate transporter/NMDAR1 status. These findings may be an indicative that GTPs protect either glial as epithelial retinal cells from the high glucose damage. Studies to identify the mechanisms of action of GTPs on different retinal cells in HG are being performed.

Supported by: Fapesp

1115

Polyphenol enriched cocoa protects the retinal function in experimental diabetes: an *in vivo* and *in vitro* study

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Background and aims: A significant body of evidence demonstrates that moderate chocolate consumption could be part of a healthy diet has gained acceptance in past years based on the health benefits ascribed to selected cocoa components. It has been demonstrated flavonoid compounds found in foods, including epicatechins found in high-cocoa-solid chocolates, in the prevention and treatment of diabetic complications by antioxidant and anti-inflammatory properties. The mechanisms behind these effects are still under investigation. The blood-retinal barrier (BRB) is essential to maintaining the eye as a privileged site and is essential for normal retinal function and diabetic retinopathy (DR) is associated with early alterations of the BRB in its pathogenesis. The objective of this study was to investigate the effects of polyphenol enriched cocoa in neural retina and in BRB function in an experimental diabetic rat.

Materials and methods: Diabetes was induced by streptozotocin (60 mg/kg) in normotensive rats (WKY) with 10 week-old. The animals were divided into 4 groups, control and diabetic WKY treated with polyphenol enriched cocoa (190 mg/kg), and control and diabetic WKY with placebo (cocoa without polyphenols) by oral gavage daily. After 16 weeks, the animals were submitted to electroretinography (ERG) and then euthanized; the retinas were collected for protein extract and morphology. The *in vitro* study was performed with human retinal pigment epithelium cells line (ARPE-19) exposed to high glucose (30 mM) for 24 hours. The cytotoxicity of the polyphenol enriched cocoa on ARPE-19 cells was determined by MTT assay and 10 ng, 100 ng and 1 µg/ml were selected for the experiments. The total intracellular reactive species (ROS) was determined by 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) method.

Results: As expected, body weight was lower and glycemia was greater in diabetic WKY's than in non-diabetic rats ($p = 0.0008$ and $p < 0.0001$ respectively); the systolic blood pressure were similar in all studied groups. An increase of inducible nitric oxide synthase (iNOS) measured by western blot was observed in diabetic placebo (DM-placebo) ($p < 0.05$) compared with non-diabetic placebo (non-DM placebo) group. The treatment with polyphenol enriched cocoa prevented this upregulation ($p = 0.02$) in diabetic group. The retinal function, evaluated by ERG, showed a decrease in *b*-wave amplitude (post-photoreceptor response) in DM-placebo group compared with non-DM placebo. The cocoa treatment in diabetic rats protected this abnormality ($p = 0.01$). In ARPE-19 cells there was a significant increasing in ROS in cells exposed to high glucose compared to normal glucose ($p = 0.01$) and the presence of the cocoa inhibited about 30% of this ROS increase. The expression of claudin-1, a tight junction protein, was decreased in cells exposed to high glucose and it was prevented in presence of cocoa ($p < 0.05$).

Conclusion: In ARPE-19 cells, the polyphenol enriched cocoa treatment prevented the increase in ROS production and the decrease of claudin-1, an indicative of maintenance of outer BRB integrity. In diabetic rats, the treatment preserved the retinal function. Further studies are being conducted in order to better clarify the possible mechanisms involved in the beneficial effects of cocoa in diabetic milieu.

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PS 100 Retinopathy: clinical

1116

Human versus an automated grading system (Retmarker) in diabetic retinopathy screening

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Background and aims: Evaluate the benefits of using automated grading system (Retmarker technology) in screening diabetic retinopathy and compare this system with the golden standard (human grading).

Materials and methods: Anonymous retinal images (2 per eye) from 1092 patients screened in 2009 and 2010 were obtained using a non-mydiatic 45-degree fixed Cannon-45NM camera on a Diabetic Retinopathy screening program run by APDP. Images were taken and graded by 3 experienced ophthalmologist. Additionally, images were evaluated by an automated grading system - Retmarker - and classified as "Disease", "No disease". Retmarker technology performs a 2-step analysis based on the detection of Microaneurysms and Lesion Activity (progression) within the macular region. Results of the automated analysis were compared with those obtained by manual grading.

Results: The Retmarker software classified, from the 1092 patients, 539 (49.4%) as having "Disease" and 553 (50.6%) as having "No disease", thus requiring manual grading. Retmarker achieves a sensitivity of 81.4% and a specificity of 52% with a negative predictive value of 98.6% and a positive predictive value of 6.5% when compared human grading based on patient referral criteria. A total of 8 cases marked by the human grading as having signs of Diabetic Retinopathy were not detected by the automated system, out of the 1092 patients (0.73%).

Conclusion: Automated grading of diabetic Retinopathy may safely reduce the burden of grading patients with and without disease in Diabetic Retinopathy screening programs. The novel two-step automated analysis system using Retmarker has the capability to reduce the human workload of grading by approximately 50.6%, while achieving a high sensitivity of 81.4%. Applying an automated program, such as the described one, an important reduction in the number of cases the expert needs to address is achieved, and thus there is an associated cost saving. Most importantly, an automated system, although not providing full detection, provides consistency by removing subjectivity.

1117

Validation of a predictive model for diabetic retinopathy progression in type-2 diabetic patients with mild NPDR

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Background and aims: To validate a predictive model for diabetic retinopathy progression in type-2 diabetic patients with mild NPDR using non-invasive examinations.

Materials and methods: Four-hundred and twelve (412) type-2 diabetic patients with mild NPDR were included in this 2-years observational and prospective study. Three hundred and seventy-four (374) patients completed the first 6-month of follow-up and underwent: color fundus photography, retinal thickness (RT) measurements and blood tests. Microaneurysm formation rate (MAFR) was computed from color fundus photographs using a new automatic method for MA earmarking (RetmarkerDR, Critical Health SA.). Increased RT maps (Stratus OCT, Zeiss Meditec Inc.) were computed using proprietary software.

Results: Three hundred and seventy-one (371) patients were included in the analysis (1 patient was lost of follow-up and 3 patients were treated with laser photocoagulation before the 6-month visit). Patients were distributed according to RT values and MAFR into 3 distinct DR phenotypes: Phenotype 1 (low MAFR and normal RT); Phenotype 2 (low MAFR and increased RT) and; Phenotype 3 (high MAFR). One hundred and fifty (150, 40.4%) patients were assigned to Phenotype 1, 116 (31.3%) to Phenotype 2 and; 105 (28.3%) to Phenotype 3.

notype 3. Clinically significant macular edema occurred during the follow-up period only in eyes from phenotype 2 (9 eyes, 7.8%) and phenotype 3 (7 eyes, 6.7%).

Conclusion: Our preliminary data confirms the existence of 3 different phenotypes of DR progression, as previously proposed, and simultaneously shows a similar distribution although using a different population.

Clinical Trial Registration Number: NCT00763802

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1118

Altered fibrin clot properties are associated with retinopathy in type 2 diabetes mellitus

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Background and aims: Development and progression of diabetic retinopathy (DR) in type 2 diabetes mellitus (T2DM) have been associated with poor glycemic control, long disease duration and some other clinical features. However, the pathogenesis of this complication is still poorly understood. Formation of dense fibrin clots resistant to lysis has been described in patients with diabetes. It is unclear whether abnormal fibrin clot properties may predispose to vascular complications, including DR, in T2DM. We tested the hypothesis that altered clot structure and function are associated with DR in T2DM patients.

Materials and methods: We included 182 consecutive European Caucasian patients with T2DM lasting for at least 5 years (mean age at examination 56.3 ± 6.52 years). There were 111 (61%) T2DM patients without diabetic retinopathy (NDR) and 71 T2DM with DR (mean age 55.7 ± 6.2 and 57.1 ± 7 years, respectively, $p = \text{NS}$). The average duration of T2DM was 7.8 ± 5.6 in the NDR and 13.4 ± 6.8 years in the DR group, respectively ($p < 10^{-7}$). We assessed plasma fibrin clot permeation using a pressure-driven system, expressed as a permeation coefficient (Ks), indicating the pore size, and the time required for a 50% decrease in clot turbidity ($t_{50\%}$) as a marker of susceptibility to fibrinolysis. All patients underwent ophthalmological examination including fundus photography. Potentially important clinical and biochemical covariables were also measured. Determinants of DR were identified using stepwise multivariable logistic regression analysis.

Results: Patients with DR had lower clot permeability than those free of this complication (Ks 7.53 ± 1.24 vs 6.15 ± 1.18 [10^{-9}cm^2], respectively; $p < .0001$). Fibrin clot degradation by tPA was slower in DR patients ($t_{50\%}$ 10.12 ± 1.24 vs 9.12 ± 1.4 min, respectively; $p < .0001$). Logistic analysis revealed associations between DR and Ks, $t_{50\%}$, fasting glucose, HbA1c, insulin and C-peptide ($p < .05$). After adjustment for these variables, as well as for age and gender, associations between Ks and $t_{50\%}$ with DR proved to be significant.

Conclusion: Our study is the first to show that formation of compact fibrin clots and impairment of clot lysis characterize T2DM patients with retinopathy. These results might be relevant for new preventive strategies of DR in T2DM.

1119

Glomerular filtration rate deterioration as the high intraocular value of the vascular endothelial growth factor is seemed to be the marker of diabetic retinopathy progression after cataract surgery

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Background and aims: Cataract surgery in diabetics is associated with the higher intraoperative complications incidence and poor visual outcome than in non-diabetics because of the diabetic retinopathy (DR) progression. Detection of the DR progression markers and preventing treatment performing are highly important. The aim of the study was to estimate the influence of the vascular endothelial growth factor (VEGF) value in the aqueous humor (AH) and glomerular filtration rate (GFR) deterioration on the DR progres-

sion after cataract surgery. The study was cross-sectional study with following prospective observation for 12 months in humans.

Materials and methods: In the study were included 120 patients with diabetes mellitus (DM) underwent the cataract phacoemulsification with intraocular posterior lens implantation. All patients were examined by ophthalmologist and to analyze glycemia, glycated hemoglobin, GFR before operation. At the operation beginning the samples of the AH were obtain to excess the VEGF value (by the enzyme-linked immunosorbent assay). There were the estimation of visual acuity (VA) using a Golovin-Sivtsev table from a 5-m distance and color fundus photography at 5th day and at 12 month after operation and the detection of neovascular complications (such as neovascular glaucoma (NG)). There was relative risk measurement, significance was proved using chi-square statistic.

Results: There was noted that than more severe DR was than VEGF level in the AH was higher. The patients with severe nonproliferative DR and proliferative DR had poor VA after operation, than patients with mild NPDR, although before operation they didn't differ by VA. Patients with high VEGF level in the AH had 9,62-fold risk ($p = 0,0004$) of DR progression (NG was developed in 25% of cases) and they had lower VA at the 12 month after operation than patients with lower VEGF level in the AH. The incidence of the NG in patients with GFR above $60 \text{ ml/min/1,73m}^2$ was 3%, in patients with GFR below $60 \text{ ml/min/1,73m}^2$ was 17%. There was VA at the 12 month similar in these groups. Reduced GFR (below $60 \text{ ml/min/1,73m}^2$) was associated with 5,9-fold risk of NG progression after cataract surgery ($p = 0,009$). There wasn't noted the correlation between GFR and VEGF value in the AH. Other parameters (high glycated hemoglobin, glaucoma presents, blood pressure, laser treatment and diabetic nephropathy) didn't achieve the statistic significant value.

Factorial analysis of the risk factors NG development after cataract surgery

Parameter	Relative risk	Odds ratio	chi-square test, p-value
VEGF value in the AH above 137,4 pg/ml	9,62	12,3	0,0004
GFR below $60 \text{ ml/min/1,73m}^2$	5,9	7,0	0,009
Glycated hemoglobin > 7,5%	3,5	4,8	0,12
Open-angle glaucoma incidence	3,09	3,6	0,09
Performed laser photocoagulation of the retina before operation	1,65	1,8	0,58
High blood pressure (>160/80 mm Hg)	1,82	1,8	0,58
Microalbuminuria/proteinuria incidence	2,8	3,1	0,12

Conclusion: Increased VEGF in the AH and reduced GFR significantly enhanced the risk of NG development after cataract phacoemulsification in diabetics.

1120

The prevalence of clinically significant macular edema in patients with type 2 diabetes: Istanbul diabetic retinopathy study - IDRS report 2

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Background and aims: To determine the prevalence of clinically significant macular edema (CSME) in people with type 2 diabetes (T2DM) who have applied to the Diabetes outpatient Clinic between the years 1998 and 2010.

Materials and methods: This study includes 5 818 patients with T2DM (F/M 2189/3629, mean duration of DM 8.9 ± 7.8 years, mean chronological age 57.3 ± 11.2 years). The patients were seen by the same ophthalmologist at their visit to the Diabetes Outpatient Clinic or were referred as a routine diabetes follow-up for fundus examination between the years 1998 and 2010. The findings at their last visit for ophthalmological examination were considered. All patients underwent a comprehensive ophthalmologic evaluation, including funduscopy using a 78 D aspheric lens with a slit lamp and an indirect ophthalmoscopic examination. In addition fundus fluorescein angiography and OCT were performed if necessary. The CSME was defined according to the Early Treatment Diabetic Retinopathy Study classification protocol or if there was a prior history of macular edema with evidence of photocoagulation treatment and fundus fluorescein angiography and/or OCT consistent with it.

Results: Diabetic retinopathy was present in 2195 patients (37.7%). Clinically significant macular edema was diagnosed in 405 patients (7.0%). The preva-

lence of CSME rose from 2.2% in patients with duration of diabetes less than 6 years to 16.0% in patients with duration of diabetes more than 15 years.

Conclusion: High prevalence of diabetic CSME in people with T2DM and especially in the group with duration of T2DM more than 15 years is noticeable. These findings support the need both for identification and modification of risk factors for the development of CSME in order to prevent it and also timely diagnosis and effective treatment for the patients who suffer from it.

1121

Psychometric validation of the NEI-VFQ 25 in patients with diabetic macular edema

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Background and aims: Diabetic macular edema (DME) is a common cause of impaired vision and blindness amongst diabetic patients. If not detected and treated early, the resulting vision loss can lead to considerable health costs and decreased health-related quality of life (HRQoL). The aim of this study was to provide evidence of the psychometric properties (i.e. reliability, validity, responsiveness, clinically meaningful change) of the National Eye Institute - Visual Functioning Questionnaire (NEI-VFQ 25) for use in a cohort of DME patients who participated in a clinical efficacy and safety trial of pegaptanib sodium (Macugen).

Materials and methods: A phase 2/3 randomised, double masked trial evaluated pegaptanib injection versus sham injection in patients with DME. The modified intent-to-treat analysis comprised 235 patients. QoL was measured using the NEI-VFQ 25 and the EQ-5D health index. The NEI-VFQ 25 was administered by a trained interviewer by telephone in the week prior to baseline and 54 weeks of treatment along with the EQ-5D health index. The NEI-VFQ 25 is a vision-specific measure of QoL composed of 8 multi-item scales, 4 single-item scales, and 1 composite score ranging in value from 0 (poor) to 100 (high HRQoL). Distance visual acuity (DVA), measured according to the Early Treatment Diabetic Retinopathy Study (ETDRS), was assessed at all time points.

Results: The overall operating characteristics of the NEI-VFQ 25 are supported. Exploratory factor analysis suggest that an 11 rather than 12 factor solution may be appropriate; however, variable clustering methods show that none of the 8 established multi-item scales met the criterion for further splitting. Internal consistency reliability was demonstrated for 7 out of 8 multi-item scales with Cronbach's alpha ranging from 0.58 (Distance Activities) to 0.85 (Vision Specific: Dependency). With the exception of ocular pain, the VFQ domains generally showed a low to moderate correlation with EQ-5D visual analogue scale (range 0.16–0.43) and visual acuity score (range 0.10–0.41). Construct validity was upheld with higher VFQ scores for patients who see more letters according to the ETDRS. All scales were shown to be responsive (except for Social Functioning) with Guyatt's statistic ranging from 0.10 to 0.56 at 54 weeks.

Conclusion: The NEI-VFQ 25 is a valid and reliable patient reported outcome instrument for use in DME. The existing 12-domain factor structure appears to be adequate though researchers may want to consider additional analyses with different datasets of DME patients to confirm this.

Clinical Trial Registration Number: NCT00605280

Supported by: Pfizer Inc

1122

Pooled safety analysis in patients with visual impairment due to diabetic macular edema treated with 0.5 mg ranibizumab in RESOLVE and RESTORE trials

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Background and aims: The pivotal trials RESOLVE (n=151, phase II) and RESTORE (n=345, phase III) have demonstrated the efficacy and tolerability of repeated intra-ocular ranibizumab injections in patients with visual impairment due to diabetic macular edema (DME), a complication occurring in 1–3% of diabetic patients. The aim of the present analysis is to evaluate the

overall relative risk (RR) of targeted adverse events (AEs) in patients treated with ranibizumab in these two controlled trials.

Materials and methods: In total 217 patients treated with 0.3–1.0 mg ranibizumab and 159 control patients treated with sham (RESOLVE) or laser photocoagulation (RESTORE) were assessed. The 12-month RR of the following targeted ocular and systemic AEs was evaluated: hypersensitivity reactions, retinal pigment epithelial tear, endophthalmitis, retinal detachment, retinal tear, cataract, intraocular inflammation, intraocular pressure (IOP) increase, vitreous hemorrhage, hypertension, non-ocular hemorrhage, proteinuria, myocardial infarction (MI), non-MI arterial thromboembolic events, venous thromboembolic events, deterioration of retinal blood flow (including central retinal artery occlusion).

Results: Relative to controls, ranibizumab treatment was associated with an increased RR [95% confidence interval] at 12 months of the following AEs: IOP increase (8.79 [2.11, 36.67]), vitreous hemorrhage (2.20 [0.45, 10.75]), non-ocular hemorrhage (1.47 [0.27, 7.9]), and arterial thromboembolic events (except MI) (1.22 [0.30, 5.04]). Endophthalmitis and proteinuria were observed in 1.4% and 0.5% patients treated with ranibizumab, respectively. There was no overall increased RR for the other targeted AEs.

Conclusion: The results of this analysis are consistent with the established safety profile of 0.5 mg ranibizumab, licensed for the treatment of visual impairment due to DME in Europe in 2011, and did not reveal any new ocular or systemic safety risks.

Clinical Trial Registration Number: NCT00284050 (RESOLVE), NCT00687804 (RESTORE)

Supported by: Pharma AG, Basel, Switzerland

1123

Addition of liraglutide improves retinal endothelial function and vascular risk profile in type 2 diabetic patients well controlled by metformin monotherapy

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Background and aims: GLP-1 receptor agonists have been introduced in the treatment of type 2 diabetes mellitus (T2DM). Beside their metabolic effects, GLP-1 agonists were shown to address vascular biology and to affect endothelial function.

Materials and methods: This two arm, parallel study, included 40 well controlled patients with T2DM (20 male, age: 56.5±6.1 years; duration of diabetes: 4.3±3.1 years; HbA1c: 6.3±0.4 %; mean±SD). Main inclusion criteria were: pretreatment with metformin on a stable dosage, HbA1c < 7.0%, age 30 – 65 years. Patients were randomized to receive additional liraglutide in a stepwise escalating dosage from 0.6 mg to 1.8 mg or to remain on metformin monotherapy. After 6 weeks (1.2mg) and after 12 weeks (1.8 mg) retinal capillary blood flow was assessed using a retinal laser doppler scanner at 670 nm (Heidelberg Retina Flowmeter, Heidelberg Engineering) at baseline and 20 seconds after flicker light stimulation. In addition, venous blood was taken for the measurement of several laboratory markers characterizing vascular and endothelial function.

Results: As shown in the table, blood glucose control and body weight declined in patients receiving liraglutide in addition to their previous metformin treatment (p<0.01), while glucose control and body weight remained unchanged in those patients remaining on metformin monotherapy. The retinal microvascular hyperaemic response to flicker light stimulation tended to increase during treatment with liraglutide within the first 6 weeks (p=0.06). Even this effect of liraglutide was somewhat attenuated after 12 weeks of treatment, it still remained detectable (p=0.07). In patients on metformin monotherapy a slight, albeit non significant, deterioration in the retinal hyperaemic response could be observed. During treatment with liraglutide, a continuous decline in ADMA, E-Selectin, PAI-1, and intact proinsulin levels was obtained (p<0.05 respectively). No such effects could be found during sustained treatment with metformin.

Conclusion: This pilot study suggests a beneficial effect for the treatment with the GLP-1 receptor agonist liraglutide on retinal endothelial function, which runs in parallel with an improvement in laboratory markers for endothelial and vascular function.

Table 1. Study Parameters at baseline and after 6 and 12 weeks of study treatment (mean±SD)

	Met	Met	Met	Lira+Met	Lira+Met	Lira+Met
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
HbA1c (%)	6.36±0.37	6.31±0.31	6.32±0.29	6.32±0.37	5.89±0.24	5.77±0.30
Weight (kg)	96.6±17.5	97.6±17.7	96.7±18.0	93.2±17.1	92.7±15.1	89.7±16.6
Retinal Blood Flow Response (%)	15.7±30.9	14.4±15.7	13.0±27.5	7.0±15.1	15.4±11.5	11.1±9.9
ADMA (μmol/l)	0.40±0.06	0.40±0.06	0.38±0.07	0.39±0.08	0.37±0.09	0.35±0.06
E-Selectin (ng/ml)	42.2±13.6	42.4±14.2	42.7±14.0	43.6±15.4	41.0±15.3	40.8±15.1
Intact Proinsulin (pmol/l)	11.3±8.5	10.8±7.5	11.2±7.5	9.0±7.2	6.4±3.0	7.0±4.8
PAI-1 (pmol/l)	988.4±502.9	not done	1218.2±571.3	861.584.3	not done	666.1±499.4

Clinical Trial Registration Number: NCT01208012

Supported by: Novo Nordisk

PS 101 Neuropathy and skin: experimental

1124

Insulin regulates tyrosine hydroxylase expression in neuronal pc12 cells

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Background and aims: Impairment of insulin synthesis or action is associated with neurodegenerative disease and with a decline in cognitive function. Recent studies show that diabetes represents a risk factor for dementia. Streptozotocin-diabetic rats have lower catecholamines levels in the corpus striatum and immunoreactive tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamines synthesis, is decreased in nigrostriatal neurons of the genetically diabetic BB Wistar rats. Moreover, insulin treatment raised TH mRNA levels in intact adrenals. Hif-1 alpha is the alpha subunit of a heterodimeric basic helix-loop-helix factor activated by ERK and PI3K in response to growth factor and hypoxia. It is known that Hif-1 alpha is able to bind TH promoter.

Materials and methods: TH protein and mRNA levels in PC12 cells were evaluated by Western blot and RT-PCR respectively. ChIP experiments were performed to evaluate Hif-1 alpha binding on TH promoter.

Results: We have evaluated insulin ability to regulate the TH levels in PC12 cells. Insulin induces IR and IRSs tyrosine phosphorylation and PKB and ERK activation in PC12 cells. Treatment of PC12 cells with 100nM insulin for 2 hours leads to a 7-fold increase in the mRNA levels of TH. TH protein levels are also increased upon 2, 4 and 6 hours of insulin treatment. Pretreatment of PC12 cells either with 50 μM LY294002, a PI3K inhibitor, for 30' or with 50 μM PD98059, a MAPK inhibitor, for 1h completely abolished insulin induced increase of TH mRNA and protein levels. Moreover, insulin stimulation increases the binding of Hif-1 alpha to TH promoter. The pretreatment of PC12 cells for 16h with 100nM chetomin, a Hif-1 alpha inhibitor, almost completely abolished insulin positive effect on TH mRNA, confirming the role of Hif-1 alpha in insulin regulation of TH expression.

Conclusion: These results suggest that insulin effect on TH mRNA is mediated by Hif-1 alpha activation through an ERK/PI3K dependent mechanism. Thus, improvement of insulin signal transduction in brain could preserve neurotransmitters content in diabetic condition.

1125

Impaired release of serotonin at the spinal cord of STZ-diabetic rats

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Background and aims: Spontaneous pain is an important complaint associated with diabetic neuropathy. It is caused by disarrangements at the somatosensory system, which seem to involve both peripheral and central mechanisms. As to the central mechanisms, we have previously shown that long-term diabetes is associated with hyperactivity of the spinal nociceptive neurons and impairments in the serotonergic neuronal population of the rostroventromedial medulla (RVM). The RVM plays a key role in pain control by sending serotonergic neuronal projections to the spinal dorsal horn. At spinal level serotonin acts by inhibiting or enhancing spinal neurotransmission, according to the receptor it binds. Considering the impairments detected at the spinal dorsal horn and RVM during diabetic neuropathy it is likely that spinal serotonergic neurotransmission is disrupted in this pain condition. The aim of the present study was to evaluate the levels of serotonin in spinal homogenates and in cerebrospinal fluid (CSF) collected from the lumbar spinal cord of streptozotocin (STZ)-diabetic rats, along with the evaluation of monoamine oxidase A (MAO A) activity, the main enzyme involved in serotonin degradation.

Materials and methods: Diabetes was induced by intraperitoneal injection of STZ in male Wistar rats. Control received equal volumes of vehicle solution. At 10 weeks post-injection, an intrathecal catheter was placed in the subarachnoid space with the tip at the level of L4-L5 spinal segments and approximately 100μl of CSF was collected. Two other groups of STZ and control rats

were sacrificed and L4–L5 spinal segments were removed and homogenised. Serotonin contents were quantified by ELISA in the spinal homogenates and CSFs and MAO A activity was evaluated in the spinal homogenates. SPSS was used for statistical analysis and data were compared by independent sample t test. Statistical significance was settled at $p < 0.05$.

Results: STZ rats presented increased levels of serotonin in spinal homogenates (STZ: 0.4 ± 0.04 ng/mg tissue; Control: 0.3 ± 0.03 ng/mg tissue), but significant decreased levels in CSF (STZ: 0.3 ± 0.11 ng/ μ g protein; Control: 7.4 ± 1.07 ng/ μ g protein). The activity of MAO A was significantly lower at the spinal cord of STZ rats when compared with the age-matched non-diabetic control animals (STZ: 1.9 ± 1.14 nmol/mg protein/h; Control: 5.8 ± 1.35 nmol/mg protein/h).

Conclusion: These results point to impairments in the mechanisms of serotonin release during diabetes. The increased levels of serotonin at the spinal cord homogenates of STZ rats could represent increased accumulation of serotonin at axonal terminals from the descending RVM neuronal projections, probably caused by the decline in the release and degradation of the neurotransmitter, as suggested by the decreased levels of serotonin at the CSFs and the reduction in MAO A activity. It is likely that the impaired release of serotonin at the spinal cord will attenuate the serotonergic inhibitory action upon spinal nociceptive transmission, which may account to the spontaneous neuronal hyperactivity at the spinal cord and exacerbated pain responses detected in STZ-diabetic rats.

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Suppression of neuropathy development in spontaneously diabetic GK rats by treatment with DPP-IV inhibitor, vildagliptin

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Background and aims: Incretin is now widely used as a new type of treatment for diabetes. The incretin effects on chronic complications of diabetes attract an attention because the prevention and inhibition of the development of complications are the final purpose of diabetes management. In this study, we explored whether long-term treatment with DPP-IV inhibitor is beneficial for the neuropathy in spontaneously diabetic GK rats, non-obese type 2 diabetic model, and if it is to clarify the mechanism.

Materials and methods: Male GK rats 4 weeks of age were treated with DPP-IV inhibitor (DI: vildagliptin) at a dose of 15 mg/kg/day orally twice a day for 18 weeks. During the observation period, body weight, blood glucose, nerve conduction velocities (NCVs) were monitored regularly. Normal Wistar rats were treated in a similar manner and untreated animals served as controls. At end, all the animals were killed, and dorsal root ganglia (DRG) and sciatic nerves were extirpated for structural observations. To explore the mechanism of how DI influences on the neuropathic changes, molecular analysis on DRG and sciatic nerve tissues was conducted.

Results: Both motor and sensory NCVs were significantly delayed in GK rats. DI treatment improved the delay to 80% and 75% of normal levels, respectively, while DI treatment suppressed postprandial hyperglycemia by 26%. Average neuronal size in DRG was reduced in GK rats compared to control animals and normalized by DI-treatment. Reduced neuropeptide expression of CGRP was also improved in DI-treated rats. RT-PCR analysis disclosed the presence of both GLP-1R and GIP-R in DRG but not in the sciatic nerve. Western blots demonstrated marked reduction of Akt and phospho-Akt expressions in DRG of GK rats which was recovered to near 70% of normal levels. The alterations of cellular signaling were associated with significant recovery of suppressed mRNA expression of arginase I, a CREB-associated axon-growth enhancer, in DI-treated GK rats.

Conclusion: Neuropathic changes in GK rats were ameliorated by DI (vildagliptin) treatment which may have directly exerted Akt-phospho-Akt pathway via activated GLP-1, resulting in promoting arginase I which plays a central role in the maintenance of peripheral axons. In addition, improvement of glucose tolerance by DI-treatment may also have contributed to the recovery of peripheral nerve changes.

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Prevention and reversal of diabetic neuropathy by treatment with ghrelin M. Nakazato;

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Ghrelin, an acylated peptide produced in the stomach, increases food intake and growth hormone (GH) secretion, suppresses inflammation and oxidative stress, and promotes cell survival and proliferation. Polyneuropathy is the most common complication of diabetes mellitus, but none of agents approved for clinical use. Ghrelin's diverse functions raise the possibility of its clinical application; clinical trials with ghrelin for anorexia nervosa, diabetic gastroparesis, and cachexia have commenced. We investigated the pharmacological potential of ghrelin in the treatment of diabetic polyneuropathy in rodents and humans. Experimental diabetic polyneuropathy was induced by streptozotocin injection in C57BL/6N mice, ghrelin receptor knockout mice (GHS-R^{-/-}), and GH deficient rats. Ghrelin or desacyl-ghrelin, which lacks the acyl modification, was administered daily for 4 weeks immediately after disease onset or 4 weeks after streptozotocin injection. Ghrelin administration did not alter food intake, body weight gain, or blood glucose levels in C57BL/6N mice when compared with mice administered saline or desacyl-ghrelin. Ghrelin administration prevented and alleviated motor and sensory polyneuropathy in C57BL/6N mice in both preventive and therapeutic studies. Ghrelin reduced the plasma concentrations of oxidative stress markers and ameliorated reductions in fiber number and fiber density of sciatic nerves. In all experiments, desacyl-ghrelin failed to show any effect. Ghrelin prevented the reduction of nerve conduction velocities in GH deficient rats, but not in GHS-R^{-/-} mice. We have completed preliminary clinical application of ghrelin to type 2 diabetic patients without insulin therapy. Ghrelin at 1 μ g/kg BW was administered iv after breakfast for 2 weeks. Ghrelin did not change food intake or body weight. Ghrelin increased plasma GH level 60 ng/ml in healthy subjects at 15 min after iv injection, while ghrelin increased it to 16 ng/ml in diabetics. Ghrelin improved motor conduction velocity of the tibial nerve, sensory conduction velocity of the sural nerve, and polyneuropathy-related symptoms in all the patients. Thus, ghrelin's effects represent a novel therapeutic paradigm for the treatment of this otherwise intractable disorder. Ghrelin also prevented and alleviated muscle atrophy in a mouse model of disused muscle atrophy. Ghrelin is highly likely to be used as a novel peptide drug for diabetes mellitus and cachexia.

Clinical Trial Registration Number: UMIN00001707, University of Miyazaki IRB230

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Effects of the 11betaHSD1 inhibitor SAR184841 on peripheral neuropathy in Zucker Diabetic Fatty rats

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Background and aims: Selective 11betaHSD1 inhibitors may provide potential therapeutic treatment for patients suffering from T2D or T2D-associated complications. Such compounds have not been reported to improve microvascular complications, such as peripheral neuropathy, while specific treatments addressing these diseases still represent an unmet medical need. The aim of the study was to evaluate the effect of SAR184841, a selective 11betaHSD1 inhibitor (human IC₅₀=4nM), on glucose homeostasis, blood lipid profile, and peripheral neuropathy in a rodent model of Type 2 Diabetes.

Materials and methods: Male Zucker Diabetic Fatty (ZDF-fa/fa) rats and their lean (fa/?) controls, 11 weeks-old at the beginning of experiments, were used in the study. Diabetic (fa/fa) animals were treated with SAR184841 (30 mg/kg/day, n=10), the antihypertensive irbesartan (~30 mg/kg/day, n=10), or the oral antidiabetic rosiglitazone (~5 mg/kg/day, n=10), in chow, and compared with a vehicle-control group (n=10). After a 4-week treatment, blood and plasma metabolic parameters were measured, and peripheral neuropathy was assessed by the in vivo measurement of motor nerve conduction velocity (MNCV) from the sciatic nerve.

Results: Rosiglitazone was the only treatment to improve blood glucose homeostasis over the 4 week-period, as illustrated by %HbA1c values (8.2 ± 0.8 for the rosiglitazone group vs 12.7 ± 1.3 for vehicle, $p < 0.01$), and a significant improvement of glucose tolerance measured after 3 weeks (AUC 0-120min: 33359 ± 4765 mg/dL*min for the rosiglitazone group vs 48180 ± 1845 mg/dL*min for vehicle, $p < 0.05$). None of the compounds significantly modified plasma insulin levels. While irbesartan (230.5 ± 26.2 mg/dL, -28% vs vehicle,

$p < 0.01$) and rosiglitazone (100.6 ± 4.2 mg/dL, -69% vs vehicle, $p < 0.01$) significantly decreased plasma triglycerides versus the control group (319.6 ± 28.5 mg/dL) at the end of the experiment, SAR184841 only showed a tendency to decrease this parameter (251.3 ± 20.2 mg/dL, -21% vs vehicle, $p = 0.05$). However, SAR184841 significantly decreased cholesterol levels after 4 weeks (164.2 ± 4.7 mg/dL for SAR184841 group vs 187.3 ± 8.2 mg/dL for vehicle, $p < 0.01$), whereas rosiglitazone and irbesartan had no effect. Interestingly, the MNCV, which was impaired in the vehicle-ZDF rats (31.8 ± 1.4 m/s vs 45.0 ± 1.3 m/s in lean animals), was significantly improved by SAR184841 (39.5 ± 2.0 m/s, $p < 0.01$ vs vehicle-ZDF), and by rosiglitazone (37.2 ± 1.2 m/s, $p < 0.05$ vs vehicle-ZDF). Irbesartan had no effect on this parameter (34.2 ± 0.7 m/s).

Conclusion: Taken together, these results show that the 11 β HSD1 inhibitor SAR184841 improved peripheral neuropathy in diabetic animals and that this effect can be achieved independently of a major effect on blood glucose control. Complementary histological studies are currently running to evaluate the structural alterations of intra-epidermal nerve fibres.

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Thermal nociceptive threshold in diabetic db/db mice: the use of loop-diuretics as analgesics

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Background and aims: Almost one third of all diabetic patients suffer from painful diabetic neuropathy (PDN). PDN is characterized by hyperalgesia i.e., increased sensitivity to noxious stimulus. The primary target of PDN is the primary sensory neuron (PSN). GABA-mediated depolarization of PSNs is a fundamental event in gating the flow of nociceptive information in the spinal cord. GABA released from interneurons mediate primary afferent depolarization (PAD) via Cl⁻ channels coupled to GABAA receptors. This is possible because PSNs maintain their [Cl⁻]_i above electrochemical equilibrium generating and outwardly directed transmembrane Cl⁻ gradient. The Na⁺K⁺2Cl⁻ co-transporter 1 (NKCC) plays a key role in the generation and maintenance of this gradient. Hence, NKCC modulates and control PSN excitability, in particular nociceptive processing, and participates in the generation and maintenance of hyperalgesic states. Genetic ablation of NKCC1 expression in mice causes impaired nocifensive (pain-avoiding) behavior. In the hot-plate test of nociception, adult NKCC1 knock-out mice show longer response latencies to noxious heat and increased behavioral thresholds after intra-dermal capsaicin injection. However, this conventional method for the study of thermal nociception in rodents applies constant supra-threshold heat stimuli (52–55°C) and measures the reflex latency of nocifensive reactions of the animal. The response latency to noxious stimuli determined by these methods vary considerably upon repeated measurements, which is a major disadvantage concerning reliability and reproducibility.

Materials and methods: By applying a slowly increasing thermal stimulus we have determined the thermal nociceptive threshold of diabetic db/db and WT mice and the results were compared to the ones obtained with mice lacking NKCC1.

Results: The thermal nociceptive threshold, that is the lowest temperature at which the animal shows nocifensive behavior, of db/db mice, WT or NKCC1 KO mice were: $47.1 \pm 0.3^\circ\text{C}$ ($n=20$), $48.0 \pm 0.1^\circ\text{C}$ ($n=64$) and $49.2 \pm 0.2^\circ\text{C}$ ($n=34$), respectively ($p < 0.001$). Injection of non-diuretic doses of bumetanide (or furosemide), both inhibitors of NKCC, to these mice resulted in a significant increase in their thermal nociceptive thresholds ($p < 0.01$): db/db $49.1 \pm 0.4^\circ\text{C}$ ($n=7$), WT $50.0 \pm 0.3^\circ\text{C}$ ($n=8$) and KO $49.8 \pm 0.2^\circ\text{C}$ ($n=8$).

Conclusion: These results suggest that diabetic db/db mice are hyperalgesic whereas mice lacking NKCC1 have higher than normal thermal nociceptive thresholds. Furthermore, these thresholds could be increased after treatment with NKCC inhibitors, confirming a partial, although not exclusive role of NKCC in the maintenance of the thermal nociceptive threshold in these mice.

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Effects of urotensin receptor antagonist SB-657510 in diabetic erectile dysfunction *in vitro*

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Background and aims: The pathogenesis of erectile dysfunction (ED) in diabetes mellitus (DM) is complex in nature, multifactorial and not understood in details. The prevalence of questionnaire-diagnosed ED in primary care is 30–50%. Urotensin-II (U-II) has been identified as an endogenous ligand for the orphan G-protein coupled receptor 14, initially named as sensorial epithelium neuropeptide-like receptor. Elevated plasma levels of U-II and increased expressions of U-II per se and its receptor (UT) have been demonstrated in numerous diseased conditions including hypertension, atherosclerosis, heart failure, pulmonary hypertension, diabetes, renal failure, and the metabolic syndrome. U-II significantly relaxes human corpus cavernosum strips in endothelium- and NO-dependent manner. The peptide caused a significant increase in intracavernous pressure in anesthetized rats although the cumulative addition of U-II was not able to produce either relaxation or additional contraction in pre-contracted cavernosal strips from rats or mice. We aimed to determine the role of UT antagonist, SB-657510 in diabetic ED.

Materials and methods: Diabetes was induced by multiple intraperitoneal streptozotocin injections (55 mg/kg/day, five days) in male Swiss albino mice. Animals were killed 6 weeks after last streptozotocin dosage. Cumulative concentration-response curves for the phenylephrine and Rho-kinase inhibitor Y-27632 (after 80% maximal phenylephrine precontraction) were determined. Cumulative responses to acetylcholine were measured to determine endothelium-dependent relaxation. Transmural electrical field stimulation (EFS) was delivered using platinum wire electrodes (duration 30 seconds, 2 to 64 Hz, 10-ms pulses, 40 V). In the presence of atropine (1 μM) and guanethidine (3 μM), nitrenergic nerve-mediated relaxation in response to electrical stimulation was assessed against phenylephrine precontraction. This protocol is repeated in both control and diabetic corpus cavernosum after incubation with 3×10^{-7} M SB-657510 for 30 minutes.

Results: Both contractile responses to phenylephrine and relaxant responses to EFS, acetylcholine and Y-27632 were increased in diabetic cavernosal strips. *In vitro* incubation of the diabetic strips with SB-657510 significantly prevented the increase in phenylephrine contractility ($p < 0.01$), whereas it further enhanced the increased relaxant responses ($p < 0.05$). In the control strips, EFS-induced relaxation was significantly attenuated after incubation with SB-657510 ($p < 0.05$).

Conclusion: This is the first study suggesting that U-II has an active involvement in the pathogenesis of diabetic ED and antagonism of UT with SB-657510 restores diabetes-induced ED. In conclusion, it is considered that U-II may be a good target in the research and development of drugs to treat diabetic ED.

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The effect of aminoguanidine on cultured human fibroblasts exposure to advanced glycation end products

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Background and aims: In diabetes, the excessive accumulation of extracellular matrix proteins and skin aging are considered to be due to advanced glycation end products (AGEs) formation. The aim of this study was to elucidate the controversial effect of aminoguanidine (AG) on the expression of AGEs receptor (RAGE) and TGF- β in relation to type I and III collagen regulation at transcriptional and translational levels in cultured fibroblasts exposure to AGE-BSA.

Materials and methods: The 70% confluent CCD-1070Sk fibroblast cultures were treated for 12 and 24 hours with 50 $\mu\text{g}/\text{ml}$ AGE-BSA in the presence and absence of 10–100 μM AG. The mRNA expression of all target genes was analyzed by qPCR. Type I and III collagen and TGF- β from conditioned media and RAGE from cell membranes were investigated by Western immunoblot.

Results: The cell treatment by 50 $\mu\text{g}/\text{ml}$ AGE-BSA up-regulated the mRNA and protein expression of RAGE, TGF- β and both procollagens $\alpha 2$ (I) and $\alpha 1$ (III). Thus, the relative expression ratio (R) increased to: 4.1 ± 0.25 for RAGE, 3.07 ± 0.32 for TGF- β , 5.85 ± 0.14 for procollagen $\alpha 2$ (I) respectively

2.73+/-0.34-fold after 12 hours and to 2.67+/-0.19, 1.81+/-0.35, 2+/-0.33 and 1.18+/-0.19 at 24 hours of exposure. After 12 hours of AGE-BSA and AG co-treatment, the relative expression ratio (R) of RAGE and TGF- β increased to 6.6+/-0.15 and 5.16+/-0.16-fold at 10 μ M AG and with the rise of AG concentration, R decreased but at 100 μ M AG it remained of 4.5+/-0.19 and 3.86+/-0.17 levels. R for procollagen α 2 (I) and α 1(III) increased with the rise of AG level and at 100 μ M AG, R for procollagen α 1(III) equalized the R for α 2 (I), being increased to 8+/-0.12. After 24 hours, R for all target genes decreased with the increase of AG concentration, but only at 100 μ M AG, R had subunitary value for RAGE and TGF- β . The relative protein expression of RAGE at 12 hours increased with AG concentration but at 24 hours decreased with AG level, reaching a subunitary value. In culture media, TGF- β had a similar profile with RAGE but its relative expression after 24 hours did not reach subunitary value. Both collagen types accumulated in time, reaching at 12 hours the same relative protein expression. After 24 hours of treatment, only the relative protein expression of type III collagen had a decreasing tendency reaching at 100 μ M AG the value recorded in the presence of 50 μ g/ml AGE-BSA.

Conclusion: Our results provide further evidence for the hypothesis that TGF- β is regulated by RAGE-AGE interaction and plays a pivotal role in mediating collagen deposition in diabetes context, especially at skin level. The positive effects of AG can be observed after 24 hours when it decreased the mRNA and protein expression of RAGE. Probably after 24 hours it could reduce the extracellular matrix accumulation of both collagen types, restoring the ratio collagen I/collagen III in the collagen's I favour.

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Diabetes impairs the expression of the CB₁ cannabinoid receptor, the novel homeostatic regulator of the skin

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Background and aims: The G protein-coupled metabotropic cannabinoid receptor type-1 (CB₁R) is a major regulator of metabolism, growth and inflammation, and is present in most tissues of the body. For the first time, we aimed to test the expression of CB₁R and other factors of inflammation and regeneration in the skin of diabetic and in CB₁R knockout mice (CB₁R KO).

Materials and methods: We quantified by q-RT-PCR markers of proliferation, inflammation, angiogenesis and oxidative stress, in the skin of wild-type (WT) control, WT streptozotocin (STZ)-induced diabetic mice and CB₁R KO mice.

Results: In the skin of the CB₁R KO mice, the expression of 1) the growth factors, epithelial growth factor (EGF), fibroblast growth factor (FGF), stromal derived growth factor (SDF-1) and its receptor, CXCR4, 2) the inflammatory markers, interleukin-6 (IL-6), keratinocyte-derived chemokine (KC), tumor necrosis factor-alpha (TNF-alpha), monocyte chemoattractant protein-1 (MCP-1) and metalloproteinase-9 (MMP9), as well as 3) the antioxidant defense enzymes, heme-oxygenase-1 (HO-1) and superoxide dismutase (SOD), were all decreased when comparing with WT mice. Furthermore, we found that CB₁R expression is decreased in the skin of diabetic mice, indicating the possible impairment of the expression of these CB₁R-dependent homeostatic markers.

Conclusion: Apparently, the absence of CB₁Rs impairs the expression of several markers involved in the control of inflammation and tissue regeneration. Thus, the observed decrease in CB₁R expression may be a major pathologic mechanism in diabetic skin complications.

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PS 102 Neuropathy: small fibres and pain

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Intraepidermal nerve fibre density is associated with weight

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Background and aims: Intraepidermal nerve fibre density (IENFD) quantification is regarded to be a sensitive and specific measure of small nerve fibre dysfunction and IENFD loss is an early feature in glucose dysregulation. Our aims were to study IENFD in individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D) and to study if IENFD was associated to metabolic traits, e.g. obesity and dyslipidemia, and to neurophysiologic assessments of nerve function.

Materials and methods: Participants were consecutively recruited from the population-based Västerbotten Intervention Program; NGT (n=22), IGT (n=14), T2D (n=24), at the age of 60±1 years. The individuals' height and weight were measured. Blood glucose and lipids were measured. Nerve conduction studies (NCS) were performed (sural and peroneal nerves) and the results were standardized to z-scores and compiled into a composite Z-score representing the nerve function in the leg. Neuropathy disability score (NDS) was used to evaluate neuropathic signs. In addition, thermal threshold tests (TTT) were performed to assess small nerve fibre function. Skin biopsies were performed using a 3-mm punch taken 10 cm proximal to the lateral malleolus. The intraepidermal nerve fibres were evaluated by routine immunohistochemistry and stained with anti-PGP9.5 (ubiquitin carboxyl-terminal hydrolase) antibodies. Light microscopy was used to identify nerve fibres in thin sections (5 μ m) according to a standardized protocol. The IENFD was given as the mean of counts in 3 sections per millimeter of epidermal length. The assessors were blinded to the identity of the samples.

Results: Patients with diabetes had lower IENFD (median 2.9 nerves mm⁻¹, IQR 1.2-4.8) than controls (median 4.4 nerves mm⁻¹, IQR 3.5-6.3; Mann-Whitney U test p=0.007). IGT individuals did not differ in IENFD (median 3.2 nerves mm⁻¹, IQR 1.4-5.5) compared to controls (p=0.12) or diabetic patients (p=0.53). IENFD was positively correlated to NCS (r=0.39, p=0.002), but not to TTT and NDS. Individuals in the 3rd tertile of composite Z-score (i.e. better nerve conduction) had higher IENFD (median 4.1 nerves mm⁻¹, IQR 2.7-5.8) than individuals in the 1st tertile (median 2.4 nerves mm⁻¹, IQR 0.7-3.9; p=0.009). Triglycerides and cholesterol were not associated with IENFD. However, a stepwise multiple linear regression analysis revealed that weight was independently associated to IENFD, after adjustment for age, sex, height, and diabetic status (β = -0.419, p<0.001).

Conclusion: We conclude that skin biopsies for IENFD quantification in thin sections is a simple useful method for assessing small nerve fibre neuropathy in individuals with diabetes. The association between weight and IENFD indicates that metabolic traits other than glucose dysmetabolism might play a role in the development small nerve fibre neuropathy.

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Corneal nerve fibre damage defined using corneal confocal microscopy in relation to tear film proteomics

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Aims: The novel technique of corneal confocal microscopy has previously identified early and significant corneal nerve damage in diabetic patients. However, the mechanistic basis of the damage has not been explored. We have therefore undertaken an analysis of differentially expressed proteins in type 1 diabetic patients with neuropathy compared to healthy controls.

Methods: 10 type 1 diabetic patients (age: 52 ± 5) with a long duration of diabetes (38 ± 4 yrs) and 5 control subjects (age: 33 ± 5 yrs) underwent assessment for neuropathy. Tear samples were collected using micro-capillary tubes and analysed by LC-MS label-free comparative peptide profiling. The data acquired was searched against a human protein database and reviewed in the OBS DECIDER database.

Results: Diabetic patients had a significant neuropathy (NDS- 8.5 ± 4.8 v 0.20 ± 0.20 , $P = 0.2$), VPT (27.9 ± 8.9 v 2.9 ± 0.4 , $P = 0.07$) and using the novel technique of corneal confocal microscopy a significant reduction in corneal nerve fibre density (18.3 ± 3.7 v 35.9 ± 2.8 , $P = 0.01$), branch density (32.8 ± 10.13 v 67.4 ± 14.4 , $P = 0.08$) and length (14.6 ± 2.1 v 25.4 ± 2.2 , $P = 0.009$). In the tear samples an initial set of 36 differentially expressed proteins were identified from 103 statistically significantly different peptides in diabetic patients with neuropathy compared to control subjects. The following proteins were particularly significantly increased: Ceruloplasmin (5.90 fold, $P = 0.02$); Cystatin-C (1.4 fold, $P = 0.03$); Galectin-3-binding-protein (5.10 fold, $P = 0.04$); Lysozyme C (8.50 fold, $P = 0.0001$); Nucleobindin-2 (7.67 fold, $P = 0.01$); SPARC-like protein 1 (4.19 fold, $P = 0.05$). The following proteins were significantly decreased: AntileukoproteinaseALP (5.09 fold, $P = 0.05$); Caskin-1 (3.89 fold, $P = 0.02$); Complement C3 (4.67 fold, $P = 0.07$); Haptoglobin (-10.82 fold, $P = 0.003$); prolactin-inducible protein (-1.39, $p = 0.005$); vonWillebrand factor A domain containing protein 3A (-7.02 fold, $P = 0.009$).

Conclusion: This analysis identifies increased tear proteins which have previously been associated with oxidative stress (plasma Ceruloplasmin), neuropathy (plasma Cystatin-C). It also identifies reduced expression of complement C3, and vonWillebrand protein which may be relevant to neuropathy as both show increased deposition in blood vessels of patients with neuropathy. However, this analysis fails to show significant differences in a range of neurotrophins and growth factors which have previously been implicated in nerve damage, despite significant corneal nerve damage observed via CCM. It also reveals potentially new candidates and target pathways for future study and intervention in diabetic neuropathy.

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Factors influencing pain experience in patients with diabetic neuropathy
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Background and aims: While the impact of diabetic painful neuropathy (DPN) on individuals' functioning and quality of life is well researched, factors influencing DPN experience are poorly understood. This study of patients with diabetic neuropathy, DN ($n = 346$; age 61.2 ± 10.8 ; 71% male; 73% type 2 DM) examined the cross-sectional and longitudinal (baseline-18 months) associations of demographic, disease and psychological factors to DPN.

Materials and methods: DPN symptom severity was assessed with a 7-item NeuroQoL-pain scale by patient self-report (2.9 ± 0.8); the Neuropathy Disability Score, NDS (total score: 7.4 ± 2.2) assessed the clinical signs of small (pain and temperature) and large (vibration and Achilles reflexes) sensory-motor fiber dysfunction; Sleep Impairment was measured with 1-item from NeuroQoL scale. Psychological self-report instruments included: the Hospital Anxiety and Depression Scale, HADS, measuring anxiety HADS-A and depression HADS-D; the Big Five Inventory, BFI, a personality measure assessing neuroticism, openness, agreeableness, conscientiousness, extraversion, and Illness Perception Questionnaire-Revised, IPQ-R, adapted to DN to assess specific cognitions of chronic timeline, unpredictable course, consequences, treatment and personal control. Multivariate linear regression tested relationships between baseline predictors in three models of DPN severity: (1) baseline, (2) follow-up, and (3) change in DPN over time.

Results: BFI-neuroticism emerged as the only personality characteristic with significant cross-sectional and longitudinal relationships to DPN severity. However, these relationships were reduced to non-significance by the addition of HADS-A and HADS-D. The temperature component of NDS was a significant positive predictor of DPN severity cross-sectionally and longitudinally, but these relationships were reduced to non-significance by the addition of HADS-D. (1) In the cross-sectional model, factors independently associated with more severe DPN were HADS-A ($\beta = 0.13$; $p = 0.020$); HADS-D ($\beta = 0.16$; $p = 0.002$); Sleep Impairment ($\beta = 0.47$; $p < 0.001$) and IPQ-R symptom unpredictability ($\beta = 0.15$; $p = 0.001$). (2) The longitudinal model was largely consistent with baseline results with younger age ($\beta = -0.10$; $p = 0.04$); HADS-A ($\beta = 0.17$; $p = 0.007$); Sleep Impairment ($\beta = 0.39$; $p < 0.001$) and IPQ-R symptom unpredictability ($\beta = 0.1$; $p = 0.026$) as independent predictors of DPN at 18 months. The strongest longitudinal predictor of DPN was baseline pain severity ($\beta = 0.72$; $p < 0.001$), explaining 62% of the variance in DPN at 18 months. (3) Change-based analyses, controlling for baseline DPN, showed that younger age ($\beta = -0.09$; $p = 0.025$) and higher levels of HADS-A

($\beta = 0.09$; $p = 0.021$) were the only significant predictors of increments in DPN over time.

Conclusion: These data suggest that although pain experience in diabetic neuropathy is consistently accompanied by depression, sleep impairment, and negative cognitive appraisals, these factors are unlikely to be causally related to pain. The most important predictor of pain over time is baseline pain severity. Anxiety may also play a causal role. Pain management in patients affected by diabetic neuropathy should therefore target both pain intensity and anxious mood.

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Phantom limb pain with diabetic amputation is no different to that of non-diabetic amputation

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Background and aims: There is a commonly-held belief that diabetic amputees experience less phantom limb pain (PLP) than non-diabetic amputees because of the effects of diabetic peripheral neuropathy. The aim of our study was to validate or refute this belief; we did this by examining the effects of diabetes on the prevalence, severity and characteristics of PLP and phantom sensations. We also explored the relationship between PLP and stump pain, and attempted to determine whether PLP had any effect on mobility.

Materials and methods: We used a customized postal questionnaire to collect information about amputees' experiences of phantom limb pain. Using the patient database from a UK regional centre for mobility rehabilitation (Lancashire Teaching Hospitals), we sent the questionnaire to 200 of the most recent lower limb amputees receiving mobility rehabilitation.

Results: From the 200 questionnaires sent, we received 102 complete responses; there were 11 respondents with amputations on both legs (who completed two questionnaires), therefore the total number of accounts of PLP was 113. Of these 113 accounts of PLP, there were 16 instances of recent trauma to the amputation stump. Limbs with recent stump trauma produced significantly higher average phantom limb pain scores (5.69 ± 0.59 out of 10 on a numerical pain scale), compared to limbs with no recent stump trauma (4.13 ± 0.29) ($p = 0.0416$). When comparing PLP prevalence and severity between the diabetic and non-diabetic groups, we excluded limbs with recent stump trauma to prevent this variable from skewing the results. Forty-four (50%) of the amputees without recent stump trauma reported that they had diabetes mellitus (DM group), while forty-four (50%) reported that they were non-diabetic (ND group). Some degree of PLP was reported in 85.6% of amputated limbs, and there was no difference in the prevalence of PLP when comparing the DM group (prevalence = 82.0%) and the ND group (prevalence = 89.4%) ($p = 0.391$). Phantom sensations were reported in 68.0% of amputated limbs, and similarly there was no difference in the prevalence of phantom sensations when comparing the DM group (66.0%) and ND group (70.2%) ($p = 0.665$). The average intensity of PLP was $3.89 (\pm 0.40)$ for the DM group and $4.38 (\pm 0.41)$ for the ND group, this was not a statistically significant difference ($p = 0.402$). To assess the impact of PLP on mobility, we asked "does phantom limb pain ever stop you walking?" The mean phantom limb pain score of the group which answered "yes" to this question was significantly higher than the remainder of the respondents, at $7.04 (\pm 0.61)$ and $3.39 (\pm 0.34)$ respectively. This difference was highly significant ($p < 0.001$).

Conclusion: Despite the common belief regarding a relationship between diabetes and phantom limb pain, we could find no evidence that diabetes has any effect on the prevalence or severity of PLP. This may be because the pathological process behind the development of PLP occurs primarily in the central nervous system. We did, however, corroborate previous evidence that phantom limb pain severity has a relationship to stump pain, by showing that people with recent stump trauma had a higher average phantom limb pain score. We were also able to show that amputees with high average phantom limb pain scores were significantly more likely to have their walking affected than those with low pain scores; this is an important result, as it stresses the value of aggressively treating phantom limb pain during mobility rehabilitation.

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Sensory profiles of neuropathic pain in painful diabetic polyneuropathy
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Background and aims: The identification of distinct sensory profiles of neuropathic pain in painful diabetic polyneuropathy (PDPN) that reflect the underlying pain-generating mechanisms, can support a mechanism-based individual therapeutic approach. The aim of this study was to describe the characteristics of neuropathic pain and to determine whether specific sensory profiles are identifiable in PDPN using screening and assessment tools of neuropathic pain.

Materials and methods: In 59 patients with clinical diagnosis of PDPN, Michigan Neuropathy Screening Instrument Questionnaire, Michigan Diabetic Neuropathy Score (MDNS), Vibration Perception Threshold (VPT), Cold (CTT) and Warm Thermal Threshold (WTT), Nerve Conduction Studies (NCS), Douleur Neuropathique en 4 Questions (DN4), and Neuropathic Pain Symptom Inventory (NPSI) were performed.

Results: Paresthesia/dysesthesia (tingling and pins and needles) and burning were the most frequent sensory descriptors reported by the patients on NPSI (96% and 87%, respectively), followed by paroxysmal pain (electric shock and stabbing), evoked pain (by brushing, pressure and cold), and deep pain (squeezing and pressure) (77%, 69%, and 67%, respectively). The highest scores were reached by NPSI dimensions of burning (5.4±2.9) and paresthesia/dysesthesia (4.9±2.5), the lowest scores by evoked pain (2.1±2.1). When looking for an internal association among the sensory descriptors of DN4 or NPSI, an association was found between electric shock and pins and needles ($\chi^2=4.60$, $P=0.032$) and between tingling and itching on DN4 ($\chi^2=5.11$, $P=0.016$), between burning pain and deep pain ($\chi^2=5.36$, $P=0.012$) and between evoked pain and paroxysmal pain ($\chi^2=5.15$, $P=0.022$) on NPSI. When looking for neurological correlates of the pain descriptors, we found that DN4 burning was associated with higher sural amplitude z score (-0.98 ± 1.19 Vs -2.41 ± 0.59 , $P=0.020$), DN4 tingling with a lower number of NCV abnormalities (4.61 ± 3.22 Vs 9.27 ± 6.87 , $P=0.007$), DN4 numbness with higher VPT (28.42 ± 11.23 Vs 19.24 ± 7.4 , $P=0.004$), and DN4 prick hypoesthesia with higher MDNS (15.00 ± 5.32 Vs 8.24 ± 4.37 , $P<0.0001$), higher WTT (42.60 ± 5.11 Vs 39.26 ± 3.84 , $P=0.022$), lower CTT (22.28 ± 8.78 Vs 26.78 ± 3.97 , $P=0.017$), and lower peroneal conduction velocity z score (-2.45 ± 0.53 Vs -1.17 ± 1.46 , $P=0.021$). NPSI burning was associated with higher CTT (26.37 ± 4.05 Vs 20.22 ± 11.52 , $P=0.014$), whereas NPSI evoked pain subscore with lower sural amplitude z score (-1.70 ± 1.05 Vs -0.07 ± 1.19 , $P=0.029$). NPSI deep pain subscore was positively related to VPT ($\rho=0.32$, $P=0.032$) and inversely related to tibial motor amplitude z score ($\rho=-0.53$, $P=0.009$); NPSI paresthesia/dysesthesia subscore was positively related to VPT ($\rho=0.30$, $P=0.039$) and inversely related to peroneal nerve amplitude z score ($\rho=-0.51$, $P=0.011$).

Conclusion: A sensory profile of burning pain and paresthesia is prevalent in PDPN patients, however, other combinations of symptoms can occur, i.e. burning pain with deep pain and allodynia with paroxysmal pain. Although the suggestion that burning pain - and not evoked pain - might be associated with preserved nerve function, identification of neurological correlates of different sensory profiles in PDPN remains unachieved.

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A functional magnetic resonance imaging study of central pain processing of co-existing depression in painful diabetic neuropathy

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Background and aims: Painful diabetic polyneuropathy (painful DPN) is a distressing and disabling condition that negatively impact on quality of life. The relationship between chronic pain and depression has been well documented in painful DPN with significant association between depression, anxiety and pain catastrophising behaviour. Pain perception is a subjective experience and can be modulated by various factors including mood disturbance. A heightened response is seen in emotional/affective brain processing structures in chronic pain patients however the exact influence of mood disturbance on pain processing in DPN is still poorly understood.

Methods: 19 right-handed subjects with painful-DPN underwent neurophysiological assessments. All subjects had neuropathic pain below the knees [mean-Likert 7.5(1.8)]. Depression assessed using the Hospital Anxiety and Depression Scale (HADS-D). Depressed patients $n=12$, non depressed $n=7$. Together with 15 age-matched healthy volunteers (HV), all subjects underwent functional magnetic resonance imaging (fMRI) during noxious heat pain application to the thigh (non-neuropathic region) versus pain-free baseline thermal stimulation in a 'boxcar' paradigm. MR examinations were performed on a 3T Phillips Acheiva. Images were analysed using SPM5 (www.fil.ion.ucl.ac.uk/SPM5).

Results: Patients with painful DPN showed an increased stimulus response in the prefrontal cortex (PFC) (Talairach coordinates 18, 40, 14mm; $p<0.001$ uncorrected) and anterior cingulate cortex (ACC, 20 32 20mm) compared to HV ($p<0.001$ uncorrected). Greater activation was seen in the cingulate cortex in painful DPN patients with depression compared to those without (-12, 4, 3 0 mm; $p<0.001$ uncorrected). No clusters of activation seen in non-depression vs. depression. A greater response was seen in the posterior cingulate cortex (16 -40 12mm; $p<0.001$ uncorrected) and parahippocampal gyrus (-36, -46, -6mm; $p<0.001$ uncorrected) in depressed vs. non-depressed patients using HADs anxiety score (HADS-A) as a covariate.

Discussion: Our results suggest the cingulate gyrus plays an important role in modulating pain perception in mood disorders. Whilst the anterior cingulate has a role in emotional processing the posterior cingulate is often characterised as 'evaluative' and associated with memory through its connections with the hippocampus. Constant interruption of neuropathic pain in patients with coexisting depression results in significant emotional distress. This may explain the increased activation of the anterior cingulate cortex. Patients with anxiety report greater number of painful symptoms and pain intensity this may be mediated by increased activation of the posterior cingulate cortex and parahippocampus. Using this novel technique we are gaining new insights into brain regions involved in abnormal pain processing in DPN and the pain modulating effects of mood disorders which may lead to potentially new targets for future therapies and pain management strategies.

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Neuropathic pain severity relates to important patient related health outcomes: implications for effective early management of painful diabetic neuropathy

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Background and aims: It is well recognised that chronic painful diabetic peripheral neuropathy (painful-DPN) results in immense loss of quality of life and significant disability. However, the relationship between pain severity and important health related outcomes is less clearly defined. The goal of this study was to assess the impact of neuropathic pain severity on mood, functionality, sleep and quality of life in patients with painful-DPN.

Materials and methods: 300 patients with the diagnosis of painful-DPN were identified from the Sheffield Teaching Hospitals diabetes register. From this pool, 44 patients (mean age, 61.5 ± 10.52 , 25 males, 10 with Type 1 diabetes and neuropathic pain duration of, 9.5 ± 6.33) have so far undergone assessment of: 1) peripheral neuropathy using Neuropathic Disability Score, 2) pain severity using the Modified Brief Pain Inventory (m-BPI-DPN) and the Neuropathic Pain Scale (NPS), 3) quality of life (QoL) and functionality using the DPN specific, Norfolk QoL Scale, 3) mood using Hospital Anxiety and Depression Scale (HADS) and 4) sleep quality and duration.

Results: Based on the m-BPI-DPN scores patients were divided into mild (score of 0-3), moderate (4-6) and severe (7-10) painful-DPN groups. With increasing pain severity significant differences (one way ANOVA) between the groups were found for: number of prescription medications ($p=0.046$), general activity ($p=0.01$), interference with work activity ($p=0.001$), walking ability ($p=0.004$), Norfolk QoL Scale ($p=0.043$) and sleep interruption ($p=0.026$). However, there was a high prevalence of anxiety and/or depression (61.5%) regardless of pain severity in this cohort of patients.

Conclusion: These findings demonstrate that neuropathic pain severity is associated with worse outcomes for important patient related outcomes including functionality, QoL and sleep quality. Therefore, early identification of painful-DPN patients and effective management of pain is crucial to combat the debilitating burden of painful-DPN.

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Amitriptyline versus alpha-lipoic acid in the treatment of painful diabetic polyneuropathy

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Background and aims: Painful diabetic polyneuropathy is a serious health problem. There is a need of a medication that is well tolerated and which provides meaningful pain relief and improved quality of life. The aim of this study was to compare the efficacy and safety of alpha-lipoic acid and amitriptyline in alleviating pain associated with diabetic polyneuropathy.

Materials and methods: In this randomized, single-blind, clinical trial 32 patients with diabetic polyneuropathy who had a pain score of at least 40 mm in a 0–100 mm VAS scale, were assigned to sequential treatment with 600 mg alpha-lipoic acid once daily intravenously and placebo orally for 3 weeks or to treatment with amitriptyline orally, at doses of 25, 50 and 75 mg and placebo intravenously for 3 weeks. The treatment was followed in ambulatory for 3 months. Patients continued the treatment with the same drug orally in previously established doses. Pain relief (SFMPQ-VAS), quality of life improvement (EuroQol EQ-5D) and occurrence of adverse events were assessed weekly during hospitalization and monthly when treated in outpatient.

Results: Upwards 50% VAS reduction was noted in 7 (44%) patients on alpha-lipoic acid and 6 (38%) patients on amitriptyline ($P = \text{ns}$). A comparable reduction in pain intensity was obtained with both drugs from the first week on ($\Delta\text{VAS} -13 \pm 13$ mm vs. -13 ± 14 mm, $P = 0.94$, respectively). The improvement in quality of life was significantly higher in patients treated with amitriptyline after 1 and 3 weeks of the observation when compared to alpha-lipoic acid (respectively: $\Delta\text{EQ}_{1\text{week}} -1 \pm 10$ vs. 9 ± 13 , $\text{EQ}_{3\text{week}} 12 \pm 20$ vs. 28 ± 21 , $P < 0.05$). The adverse events were reported only with amitriptyline, dry mouth being the most common.

Conclusion: Amitriptyline and alpha-lipoic acid is similarly effective in the treatment of painful diabetic polyneuropathy. Amitriptyline has greater beneficial impact on the quality of life, but is associated with a higher risk of adverse effects.

Clinical Trial Registration Number: KIW/0022/KB1/144/I/08

PS 103 Cardiovascular autonomic neuropathy

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Reduced heart rate variability in youth with type 1 diabetes: the SEARCH CVD Study

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Background and aims: Reduced heart rate variability (HRV) is the earliest sign of cardiac autonomic neuropathy (CAN), a major diabetes complication. We examined differences in HRV markers reflecting early (parasympathetic loss with sympathetic override) or more advanced (sympathetic and parasympathetic loss) subclinical CAN in 237 youth with T1D (age 19.5 ± 2.8 years, T1D duration 10.1 ± 3.9 years) compared to 125 non-diabetic control subjects (age 19.2 ± 3.0 years) who participated in the SEARCH CVD study in Colorado and Ohio.

Materials and methods: Resting HRV measures were obtained using the SphygmoCor Vx (AtCor Medical, Lisle, IL). Body mass index (BMI), blood pressure (BP), fasting lipids (LDL and HDL-cholesterol, triglycerides) and hemoglobin A1c were measured. General linear models were used to explore group differences (T1D vs. controls) in measures of HRV, after adjustment for covariates.

Results: Older age, male sex, higher LDL-cholesterol and triglyceride levels were consistently associated with worse HRV parameters, regardless of case/control status. Compared with controls, T1D youth had lower HRV parameters in both time [standard deviation of normal RR intervals (SDNN), root mean square successive difference (RMSSD), percent of normal RR intervals less than 50 msec (pNN50)] and frequency domains [low and high frequency power (LF, HF)], even after adjustment for age, sex, race, BMI, BP and lipid levels (Table). On further adjustment for A1c, all differences became non-significant. A pattern of combined parasympathetic and sympathetic loss (reduced pNN50, RMSSD, HF with higher LF: HF ratio) reflecting a more advanced CAN stage was evident in T1D youth.

Conclusion: These findings advocate for CAN screening in youth with T1D and suggest the need for improved glycemic control to prevent the development and progression of CAN.

Adjusted HRV parameters among T1D and controls*

HRV parameters	T1D	Controls	p-value
SDNN (msec)	63.3	76.3	0.002
RMSSD (msec)	59.2	75.2	0.002
pNN50 (%)	41.5	47.1	0.03
HF power (Hz)	56.4	61.3	0.04
LF: HF ratio	1.11	0.82	0.06

* Adjusted for age, sex, race, BMI, BP, LDL-cholesterol, HDL-cholesterol and triglyceride levels

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Spectral analysis of heart rate variability: an early biomarker of cardiac autonomic neuropathy?

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Background and aims: Cardiac autonomic neuropathy (CAN) is a serious complication of diabetes and carries up to a five-fold increased risk of mortality. However, early detection of CAN is problematic since conventional autonomic function tests (AFT) are cumbersome and are not feasible in busy diabetes clinics. The aim of this ongoing study is to assess the diagnostic util-

ity of spectral heart rate variability (SHRV), which is a simple 5-minute test, as an early biomarker of CAN. Early detection of this disorder might lead to a better prognosis by the deployment of multi-factorial interventions which may slow or even reverse its progression.

Materials and methods: At baseline, 130 type 1 diabetes (T1DM) subjects underwent SHRV, baroreceptor sensitivity testing (BRS) and AFT (O'Brien's protocol). Based on the results of these tests patients were divided into no-CAN (normal BRS and AFT), subclinical-CAN (normal AFTs but abnormal BRS) and established-CAN (abnormal AFT and BRS). Using a discriminant function analysis model, we were able to correctly classify 79% of cases using only SHRV parameters. Having excluded those with established disease at baseline, 20 subjects (11 with subclinical-CAN and 9 with no-CAN) have thus far undergone the same autonomic function tests (AFT, BRS and SHRV). The mean follow-up period was 2.95 ± 0.7 years. HbA1c at initial and follow-up visit was 8.7 ± 0.39 and $8.6 \pm 0.26\%$ respectively. There were no significant changes in the use medications that would alter autonomic function between the two study periods.

Results: Three subjects with subclinical-CAN progressed to develop established-CAN (27.3% incidence rate). No subjects with no-CAN developed established-CAN over this period. There was a trend for deterioration in SHRV parameters in all subjects [mean change from baseline TP $0.09(0.52)$]. SDNN deterioration was greater in the subclinical-CAN group compared to no-CAN [subclinical-CAN vs no-CAN; $0.03(0.10)$ vs $-2.86(0.31)$; Mann-Whitney $p < 0.001$]. Subjects with subclinical-CAN who progressed to develop established-CAN demonstrated much greater deterioration in TP and SDNN compared to those who did not [TP $0.24(0.28)$ vs $-0.007(0.23)$ and SDNN $0.05(0.13)$ vs $0.02(0.10)$ respectively].

Conclusion: The preliminary results of this ongoing prospective study show an incidence for established-CAN in subjects with subclinical-CAN of 27.3% over the follow-up period. There was also a general deterioration in SHRV measures over time and this decline was most pronounced in subclinical-CAN subjects, especially those who progressed to develop established-CAN. SHRV, a simple, non-invasive and quick bedside 5-minute test that is feasible in the context of a busy diabetes clinic and seems to be an early biomarker for the development of clinically significant CAN.

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Cardiovascular autonomic neuropathy and subclinical cardiovascular disease in normoalbuminuric type 1 diabetic patients

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Background and aims: Cardiovascular autonomic neuropathy (CAN) is associated with increased morbidity and mortality in diabetes. The underlying mechanisms are largely unknown. The aim of the present study was to investigate the potential association between CAN and subclinical coronary atherosclerosis and prognostic markers of cardiac disease in asymptomatic normoalbuminuric type 1 diabetic patients.

Materials and methods: Fifty-six normotensive, normoalbuminuric, type 1 diabetic patients were divided into 26 with (+) and 30 without (-) CAN according to tests of their autonomic nerve function. Coronary artery plaque burden and calcium score (CACS) were evaluated using multislice computed tomography. Left ventricular function was evaluated with Tissue Doppler Imaging (TDI) echocardiography. Blood pressure and electrocardiography were recorded through 24 hours to evaluate nocturnal drop in blood pressure (dipping), pulse pressure and ventricular ectopia.

Results: In patients +CAN compared to -CAN there was a trend towards a higher prevalence of coronary plaques and flow limiting stenosis (24% vs. 10%), but this was not significant. However, in +CAN the CACS was significantly higher (median±range) (197 (0–5552) vs. 5 (0–312), $P=0.0012$) and only patients +CAN had CACS >400 ($n=9$ vs. $n=0$, $p=0.0004$), a value strongly associated with increased risk of cardiovascular disease. TDI of mitral annular motion showed a decrease in both early diastole (e') (7.9 (5–11) vs. 9.8 (8–13) cm/s, $p=0.0007$) and in systole (s') (7.0 (6–11) vs. 8.1 (6–11) cm/s, $p=0.028$) in +CAN compared to -CAN, demonstrating subclinical impairment in left ventricular diastolic and systolic function. Non-dipping was more prevalent (73% vs. 37%, $p=0.02$) and pulse pressure was increased (mean±standard de-

viation) (58 ± 9 vs. 46 ± 9 mmHg, $p < 0.0001$) in +CAN compared to -CAN. In multivariable analysis including age, sex, diabetes duration, HbA1c, BMI, total cholesterol and smoking, CAN was an independent predictor of increased CACS, subclinical left ventricular dysfunction, increased pulse pressure and ventricular ectopia.

Conclusion: CAN in asymptomatic normoalbuminuric type 1 diabetic patients is associated with distinct signs of subclinical cardiovascular disease. Although such findings do not explain the excess mortality reported in CAN, they do identify pathophysiological mechanisms which - if uninterrupted - could cause clinically evident cardiovascular disease.

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Effect of cardiovascular autonomic neuropathy on hypoglycaemia unawareness, QTc interval and exercise tolerance in type 1 diabetes mellitus

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Background and aims: The patients of type 1 DM with cardiovascular autonomic neuropathy (CAN) have a more risk of prolonged QTc, exercise intolerance, sudden death and poor hypoglycemic sympatho-adrenal responses. We investigated the influence of diabetic autonomic dysfunction on QTc lengthening, exercise tolerance in type 1 DM with hypoglycemia unawareness.

Materials and methods: The 80 DM1 patients (35.7 ± 1.3 y.o.) were submitted to CGM during 72-h. CAN was assessed by five cardiovascular autonomic tests by Ewing; QTc and QTcd were measured before and after treadmill. Treadmill was performed using Gardner protocol in 46 patients.

Results: Patients were divided into 3 groups: 1 - with symptomatic hypoglycemia (42,5%), 2 - with 1-2 episodes of hypoglycemia unawareness (35%), 3 - with 3 and more episodes of hypoglycemia unawareness (22,5%) during CGM time. Presence of CAN was 73 (91,25%) patients. CAN differed statistically between 1 and 2(3) groups ($p < 0,01$). Relation between CAN and hypoglycemia unawareness ($r=0,76$, $p < 0,001$), CAN and QTc ($r=0,73$, $p < 0,01$) were found. CAN differed before and after treadmill in every group and was greater after treadmill ($p < 0,01$). QTc showed a significant correlation with hypoglycemia unawareness ($r=0,71$, $p < 0,001$). QTc and QTcd were increased in 3rd group ($447,1 \pm 2,3$ ms and $106,8 \pm 34,6$ ms). After treadmill QTc differed between 1(2) and 3rd group ($p < 0,001$) and prolongation of QTc increased after exercise test. QTcd increased in 2nd and 3rd groups after treadmill.

Conclusion: CAN is related to prolonged QTc and QTcd. The patients with hypoglycemia unawareness characterized significant prevalence of autonomic dysfunction, exercise intolerance, prolonged QTc, QTcd.

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Heart rate variability is severely impaired among type 2 diabetic patients with hypertension

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Background and aims: Cardiovascular autonomic neuropathy is a common complication of diabetes mellitus and it may be present among patients with essential hypertension as well. The aim of our study was to assess heart rate variability among Type 2 diabetic patients with and without hypertension and in hypertensive patients without diabetes.

Materials and methods: Fourty type 2 diabetic, normotensive patients (mean age: $52,8 \pm 8,1$ years) 63 type 2 diabetic patients with hypertension (mean age: $55,2 \pm 6,5$ years), 74 nondiabetic patients with essential hypertension (mean age: $53,0 \pm 12,1$ years), and 25 healthy control subjects (mean age: $52,2 \pm 9,2$ years) were studied. Autonomic function was evaluated by 24 hour heart rate variability (HRV) measurement. HRV was characterized by the triangular index value (HRVti) and by the spectral components of the frequency domain analysis: the low (LF) and the high frequency (HF) components and the total power (TP).

Results: According to the two-factor analysis of variance, hypertension and diabetes were independently associated with diminished heart rate variability and their effects proved to be additive. All HRV parameters were diminished

in Type 2 diabetic patients with and without hypertension and in hypertensive diabetic patients compared to healthy control subjects, but significant difference was found only between Type 2 diabetic patients with hypertension and healthy controls. (mean±SD/ TP: 3 ± 0.4 vs. 3.4 ± 0.25 ms² $p<0.001$, LF: 2.3 ± 0.51 vs. 2.87 ± 0.28 ms² $p<0.0001$, HF: 1.96 ± 0.4 vs. 2.4 ± 0.43 ms² $p<0.01$, HRVti: 1.38 ± 0.18 vs. 1.51 ± 0.23 $p<0.05$, respectively). Moreover, the HRV parameters of Type 2 diabetic patients with hypertension were significantly diminished compared to hypertensive patients without diabetes. (TP: 3 ± 0.4 vs. 3.27 ± 0.33 ms² $p<0.001$, LF: 2.3 ± 0.51 vs. 2.68 ± 0.32 ms² $p<0.0001$, HF: 1.96 ± 0.4 vs. 2.3 ± 0.42 ms² $p<0.001$, HRVti: 1.38 ± 0.18 vs. 1.51 ± 0.14 $p<0.001$, respectively). Furthermore, the low frequency component, which is one of the most reliable predictors of poor prognosis, was significantly lower in the diabetic patients with hypertension compared to diabetic patients without hypertension. (2.3 ± 0.51 vs. 2.59 ± 0.5 ms² $p=0.01$).

Conclusion: Diabetes seems to have a greater impact on autonomic dysfunction compared to hypertension. Patients suffering from both diabetes and hypertension are at the highest risk of reduced heart rate variability. Early assessment of the autonomic nerve function is suggested being performed among diabetic patients with hypertension.

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Relation between sleep-disordered breathing and cardiac autonomic neuropathy in non-obese Japanese subjects with type 2 diabetes

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Background and aims: Although obesity is a major risk factor for sleep-disordered breathing (SDB), many Japanese subjects with diabetes are less obese than Caucasians. We evaluated the relationship between SDB and clinical parameters, especially in relation to cardiac autonomic neuropathy (CAN) in Japanese subjects with type 2 diabetes.

Materials and methods: The study included a total of 261 consecutive Japanese subjects with type 2 diabetes (161 men and 100 women, aged 40–79 years) including nonobese subjects (defined as body mass index (BMI) <25 kg/m² for Japanese) (n=141). SDB was defined by 4% oxygen desaturation index (ODI) level of 5 or more events per hour, which was measured by nocturnal pulse oximetry. CAN was examined with the variation of R-R intervals (CVRR).

Results: The patients with SDB were 24.5 and 16.3% of total subjects and nonobese diabetic subjects, respectively. The nonobese subjects with SDB had significantly lower coefficient of CVRR than those without SDB. Multiple regression analysis revealed that BMI and heart rate were significant independent factors for SDB as a whole, but CVRR was the only independent factor for SDB in nonobese subjects with type 2 diabetes.

Conclusion: SDB is highly prevalent in Japanese subjects with type 2 diabetes, even in nonobese ones, and that CAN is associated with SDB.

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Elevation of serum high molecular weight adiponectin in patients with type 2 diabetes and orthostatic hypotension: association with arterial stiffness and hypercoagulability

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Background and aims: Orthostatic hypotension (OH) is a hallmark of diabetic autonomic neuropathy and is associated with increased mortality. The serum level of adiponectin is elevated in patients with heart failure or renal failure. In the present study, we measured serum levels of total and high molecular weight (HMW) adiponectin in patients with type 2 diabetes and OH. We also investigated the relationship between the presence of OH and various clinical parameters in patients with type 2 diabetes.

Materials and methods: We studied 105 patients with type 2 diabetes. OH was defined as a decrease of 20 mmHg or more in systolic blood pressure (BP) and/or 10 mmHg in diastolic BP when BP was measured for 3 minutes while standing. The brachial-ankle pulse wave velocity (baPWV) was also measured as an index of arterial stiffness.

Results: OH was found in 30 patients with diabetes (28.6%). The hematocrit and estimated glomerular filtration rate were significantly lower in patients

with OH than in those without it. BaPWV and serum total and HMW adiponectin were significantly higher in patients with OH than in those without it. Furthermore, the HMW/total adiponectin ratio was higher in patients with OH than in those without it and hypertension was more common in patients with OH. Plasma prothrombin F1+2, a coagulation maker, was higher in patients with OH than in those without it, while there were no differences of fibrinolytic markers between the two groups. Multivariate analysis showed that HDL cholesterol, hematocrit, F1+2, baPWV, and a decline of systolic BP on standing were independent determinants of HMW adiponectin.

Conclusion: Patients with type 2 diabetes and OH had an elevated serum level of HMW adiponectin, which was associated with the simultaneous presence of renal dysfunction, anemia, arterial stiffness, and hypercoagulability.

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Application of thioctic acid in type 2 diabetic patients with cardiovascular autonomic neuropathy and chronic heart failure

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Background and aims: Cardiovascular autonomic neuropathy seems one of the pathogenetic factors of heart failure in diabetic patients. Thioctic acid proves efficiency in treatment of diabetic neuropathy. It has to be checked whether thioctic acid can prevent deterioration of heart failure in diabetic patients with cardiovascular autonomic neuropathy.

Materials and methods: Open prospective controlled randomized study was performed. 50 type 2 diabetic patients with cardiovascular autonomic neuropathy and chronic heart failure were included. SDNN < 33 ms (in the research of heart rate variability at 5-minute registration of an electrocardiogram in rest in patients with sinus rhythm) was regarded as criterion of cardiovascular autonomic neuropathy. The severity of heart failure was estimated by a 6-minute walk test and by the echocardiography. Glycemic control was also searched. Patients were randomized into 2 groups (27 patients in the group of intervention and 23 in the group of control). Oral thioctic acid 600 mg every day in the course of 3 months was prescribed for patients from group of intervention only. The patients of both groups were examined in 3 and 6 months after including in study. The results are presented in median and interquartile range.

Results: Level of SDNN rose in the group of intervention: 18 ms [15; 22] on basic visit, 28 ms [19; 35] in 3-d month, and 30 ms [14; 36] in 6-th month ($p < 0.001$, Friedman ANOVA). Level of SDNN in the group of control was reduced: 18 ms [14; 26] - 15.5 ms [11; 23] - 13 ms [11; 19] ($p < 0.001$, Friedman ANOVA). We did not find significant changes of glycated hemoglobin and parameters of echocardiography in both groups during 3 and 6 months. Results of a 6-minute walk test significantly went down only in control group: from 295 m [180; 324] on basic visit to 274 m [220; 294] in 3-d month, and up to 240 m [180; 299] in 6-th month ($p=0.02$, Friedman ANOVA). In contrast to control, there was not significant reduction in results of a 6-minute walk test in the group of intervention: 295 m [178; 316] - 286 m [202; 345] - 273 m [166; 370] ($p = 0.5$, Friedman ANOVA).

Conclusion: Thioctic acid 600 mg per os every day in the course of 3 months in type 2 diabetic patients with cardiovascular autonomic neuropathy and chronic heart failure improves parameters of heart rate variability and has a beneficial influence on tolerance to physical exercise.

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Decreased baroreflex gain more strongly predicts microalbuminuria and increased pulsatile stress than decreased RR E/I ratio in patients with type 1 diabetes

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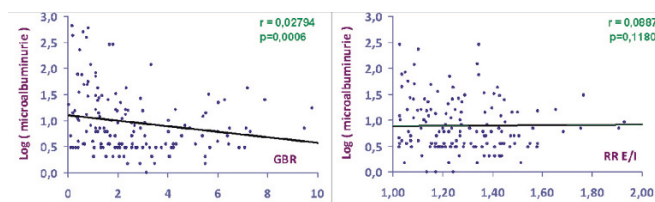
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Background and aims: Long-lasting type 1 diabetes (T1DM) may be associated with cardiac autonomic neuropathy (CAN), increased pulse pressure (PP) or pulsatile stress, and microalbuminuria (μ A), all cardiovascular risk factors. We compared the relationships of two markers of CAN, RR E/I (Expiratory/Inspiratory) ratio and baroreflex gain (BRG), with μ A and pulsatile stress during an active orthostatic test in patients with T1DM.

Materials and methods: 167 patients with T1DM (mean age 40 years, diabetes duration 20 years, body mass index 23.6 kg/m^2 , HbA1c 8.64%), who had a measurement of μ A and RR E/I ratio during a deep breathing test, were submitted to a postural squat-stand test with a continuous noninvasive arterial blood pressure (BP) monitoring (Finapres®). The mirror changes in heart rate and systolic BP during the squat-stand transition allows the calculation of a so-called BRG (msec.mm Hg^{-1}), by plotting the pulse intervals (RR) against systolic BP values, as classically assessed during a pharmacological test using the infusion of a vasodilator and a vasopressor agent. Pulsatile stress was defined as the product of PP and heart rate, both during the whole test and during the squatting position only. The T1DM cohort was divided in two subgroups according to the median value of RR E/I ratio (>1.25 vs <1.25) or of BRG (>2.20 vs $<2.20 \text{ bpm.mmHg}^{-1}$).

Results: Compared to T1DM patients with high BRG ($n = 82$; $4.51 \pm 2.31 \text{ bpm.mmHg}^{-1}$), patients with low BRG ($n = 85$; $1.17 \pm 0.61 \text{ bpm.mmHg}^{-1}$; $p < 0.00001$) tended to be slightly older (42 vs 38 years, NS), to have a slightly longer duration of diabetes (21 vs 18 years; NS), and to have lower RR E/I ratio (1.25 vs 1.31; NS), but had similar recent HbA1c levels (8.64 vs 8.64 %). However, T1DM patients with low BRG had an increased pulsatile stress index (5190 vs $4521 \text{ mmHg.min}^{-1}$; $p = 0.0019$), especially in squatting position (5408 vs $4396 \text{ mmHg.min}^{-1}$; $p < 0.0001$). Similarly, μ A was higher in T1DM patients with low BRG, being expressed by the mean level (59 ± 133 vs $10 \pm 16 \text{ mg/l}$; $p = 0.0019$) or by its logarithm to adjust for a non Gaussian distribution (1.14 ± 0.67 vs 0.76 ± 0.42 ; $p = 0.0001$). There was an inverse correlation between BRG and log μ A ($r = -0.28$; $p = 0.0006$), but not between RR E/I ratio and log μ A ($r = 0.09$; $p = 0.12$). Altogether, 26.9 % of T1DM patients with low BRG had abnormal μ A ($\geq 30 \text{ mg/l}$) versus only 5.3 % of patients with high BRG ($p < 0.001$). Similarly, the correlation between BRG and pulsatile stress ($r = -0.28$; $p = 0.0003$) was stronger than that between RR E/I ratio and pulsatile stress ($r = -0.19$; $p = 0.0153$).

Conclusion: The calculation of BRG during a squat-stand test in subjects with T1DM allows to better detect patients with increased pulsatile stress and even more strongly patients with μ A than the classical RR E/I ratio CAN index. Decreased BRG may be used to detect T1DM patients at high risk of cardiorenal complications.



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Autonomic nervous system and renal function assessment in type 1 diabetes

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Background and aims: This study investigates if there is an association between glomerular filtration rate (GFR) measurement, as index of renal function, and autonomic function assessment in patients with type 1 diabetes mellitus (T1DM).

Materials and methods: Forty-six patients (28 male, 18 female), aged 36 ± 10 years (range 19–62), with a duration of T1DM 19 ± 6 years (range 7–31), without known diabetic complications and receiving only insulin were enrolled prospectively. Participants were evaluated clinically for autonomic dysfunction with the mean circular resultant (MCR), the Valsalva maneuver (Vals), postural index (PI) and orthostatic hypotension (OH) assessment. The number of abnormal clinical tests was also considered. Within one month patients underwent glomerular filtration rate measurement with ⁵¹Cr-EDTA (expressed in ml/min/1.73m^2) and cardiac ¹²³I metaiodobenzylguanidine (MIBG) imaging [with the ratio of the heart to upper mediastinum count density (H/M) at 4 hours post-injection calculated].

Results: The values of examined variables were as follows [mean \pm 1SD (range)]: GFR 98 ± 17 (61–137), MCR 40 ± 29 (5–108), Vals 1.55 ± 0.30 (1.11–2.29), PI 1.33 ± 0.17 (1.03–1.81), OH 5 ± 9 (0–30) and H/M 1.65 ± 0.20 (1.30–2.34). GFR correlated significantly with age ($r = -0.377$, $p = 0.011$), the duration of diabetes ($r = -0.514$, $p = 0.000$), Vals ($r = 0.377$, $p = 0.010$), OH ($r = -0.448$, $p = 0.002$) and the number of abnormal clinical tests ($r = -0.366$, $p = 0.012$). MCR, PI and the H/M showed no significant correlation with GFR. There was also no significant difference in GFR between male and female patients. In multiple backward regression analysis (in which variables were entered if they were significant in univariate analysis) only the duration of the disease was found to be an independent predictor of GFR.

Conclusion: In T1DM patients GFR is associated with clinically evaluated autonomic function (which predominantly address the parasympathetic system) but not with cardiac MIBG measurements (which reflect sympathetic dysfunction). However, once the duration of T1DM is taken into account it is likely that this association is no more significant.

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Exploring prevalence and risk factors for sexual dysfunction in Dutch men and women with type 2 diabetes

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Background and aims: Diabetes is known to have a negative impact on sexual function in men. Sexual function in women with diabetes, however, has received much less attention and if addressed most studies have exclusively been conducted in women with type 1 diabetes. It has been suggested that in men with diabetes, sexual dysfunction is linked to somatic factors, whereas in women with diabetes psychological factors are more dominant. The aim of this study is to determine the prevalence of sexual dysfunction and study the risk factors of sexual dysfunction in men and women with type 2 diabetes.

Material and methods: A cross-sectional survey study was conducted in patients with type 2 diabetes, aged 40 to 75 years. In total 700 questionnaires were distributed to type 2 diabetes patients of four different diabetes centres in The Netherlands. The Female Sexual Function Index (FSFI) and the International Index of Erectile Function (IIEF) were used to evaluate sexual function. The CES-D was used to evaluate depressive symptoms. Complications were assessed based on self-report. Prevalence of sexual dysfunction was evaluated separately for men and women with type 2 diabetes. Logistic regression analyses were performed to determine which risk factors were associated with sexual dysfunction. Additional subgroup analysis was performed, all adjusted for age.

Results: In total 205 questionnaires were returned (response rate 29%). Mean age of the patients was 62.7 ± 8.1 years, 57.1% was male. Based on pre-defined criterion scores, 68.5% of men and 70.0% of women reported sexual dysfunction. Logistic regression analyses revealed three variables which were associated with sexual dysfunction in men and women with type 2 diabetes: higher age, a depression score ≥ 16 and ≥ 1 diabetes related complications (see Table 1). Depression showed the strongest association with sexual dysfunction and this association remained statistically significant when adjusted for age and diabetes related complications. The association between depression and sexual dysfunction was even stronger in men (OR= 9.67) than women (OR= 6.87) after correction.

Conclusion: Sexual dysfunction is highly prevalent in men and women with type 2 diabetes. Age, depression and diabetes related complications are associated with sexual dysfunctions in women as well as in men with type 2 diabetes. Men with depression had an almost 10-fold higher risk for sexual dysfunction and women a 7-fold higher risk than patients without depression. However it remains unclear whether depression causes sexual dysfunction or sexual dysfunction causes depression.

Table 1. Risk factors for sexual dysfunction in men and women with type 2 diabetes

	Women	Men
	Risk of sexual dysfunction	Risk of sexual dysfunction
<i>Model 1 (Univariate tests)</i>		
Age (10 year period)§	2.74 (1.21 - 6.21)**	2.00 (1.19 - 3.38)**
Depression (CES-D score ≥ 16)†	10.89 (1.27 - 93.07)**	4.51 (1.25 - 16.30)**
Complications‡	5.57 (1.48 - 21.02)**	2.52 (1.01 - 6.24)**
<i>Model 2</i>		
Depression corrected for age	7.43 (0.83 - 66.51)*	6.87 (1.77 - 26.63)**
<i>Model 3</i>		
Depression corrected for age and complications	6.87 (0.73 - 64.41)*	9.67 (1.80 - 51.86)**

Data are Odds ratio's (95% CI); CES-D score range 0 - 60; Sexual dysfunction is measured on the Female Sexual Function Index for women and the International Index of Erectile Function for men;

§ Age is divided in 10-year periods, reference is a 10 year younger person; † Reference category is no depression; ‡ Reference category is no complications; ** $P \leq 0.05$; * $P \leq 0.10$

Clinical Trial Registration Number: NTR2367

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Cardiovascular, neurological and psychological correlates of sexual dysfunction in diabetic women

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Objective: Female Sexual Dysfunction (FSD) has been described in diabetic women, but discrepancies exist between different reports. The aim of this study was to assess the prevalence of sexual dysfunction in women with diabetes mellitus, and to evaluate the metabolic, neurological, cardiovascular, and psychological correlates.

Methods: We studied 100 pre-menopausal women, in stable sex relationship, during follicular phase: 40 with diabetes mellitus (12 T1DM and 28 T2DM, without chronic complications) and 60 age-matched healthy women as controls. Sexual function, depression, and somatic neuropathy were assessed by Female Sexual Function Index questionnaire (FSFI), Beck Depression Inventory (BDI) and Diabetic Somatic Neuropathy Score (DSN), respectively. All women were evaluated for intima-media-thickness (IMT), endothelial vascular function (flow mediated dilatation (FMD), ECG (heart rate and Qtc, indexes of sympathetic activity), echocardiogram, and electromyography (amplitude and conduction of peroneal, posterior tibial, and sural nerves). Fasting blood samples were taken to measure glucose, insulin, fibrinogen, cholesterol (total, HDL-, LDL-), triglycerides, HbA1c, HS-PCR, PRL, testosterone and estradiol. Finally, we evaluated physical activity and smoking habits, parity, weight, BMI and waist circumference (WC).

Results: Score for lubrication and orgasm domains was statistically lower in diabetic women than in control group ($P < 0.05$), as well as nerve conduction and amplitude ($p < 0.05$, $p < 0.001$); diabetic women showed significantly higher BDI and SDN Score ($p < 0.01$, $p < 0.04$, respectively). In all women considered together, FSFI score was directly correlated with echocardiographic

Doppler E waves ($r = 0.24$), physical activity ($r = 0.24$) and with peroneal nerve amplitude ($r = 0.21$), and inversely with parity ($r = 0.298$), age ($r = 0.23$), BDI ($r = 0.43$), SDN ($r = 0.35$), IMT ($r = 0.31$), testosterone ($r = 0.2$), HbA1c ($r = 0.23$), ($P < 0.05$ to $P < 0.01$).

Conclusions: The findings of this study indicate that diabetes, even in the absence of complications, significantly affects sexual function in women. Although depression is the major predictor of FSD, decreased FSFI is associated with sub-clinical vascular and neurological impairment.

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Treatment of erectile dysfunction in men with diabetes: results of a phase 3, multicenter, randomised, controlled trial of avanafil

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Background and aims: Erectile dysfunction (ED) is present in >50% of men with diabetes, since comorbidities associated with diabetes (eg, neuropathy, impaired circulation) can interfere with normal erectile function (EF). Treatment with phosphodiesterase type 5 (PDE5) inhibitors currently constitutes first-line therapy. Avanafil is an investigational, rapidly absorbed PDE5 inhibitor with high PDE5 specificity. This study prospectively assessed the safety and efficacy of avanafil for the treatment of ED in adult males with type 1 or type 2 diabetes.

Materials and methods: This multicenter, double-blind, study randomized 390 eligible subjects (type 1 [10.5%] or type 2 [89.5%] diabetes; mild-to-severe ED of ≥ 6 months' duration) to placebo, avanafil 100 mg, or avanafil 200 mg for on-demand use for 12 weeks. Sexual function was assessed using patient diaries and the International Index of Erectile Function (IIEF) questionnaire. Coprimary end points included changes in successful vaginal insertion (Sexual Encounter Profile [SEP2]); successful intercourse (SEP3); and the EF domain score of the IIEF (IIEF-EF). There were no restrictions on the timing of food or alcohol intake, and concurrent alpha-blocker use was permitted.

Results: Least-squares (LS) mean change from baseline in the percentage of successful insertions and intercourse (SEP2 and SEP3, respectively) and in the IIEF-EF domain score were significantly improved following administration of both 100-mg and 200-mg doses of avanafil relative to placebo ($P \leq 0.002$ for both comparisons) (Table). The change from baseline in percentage of attempts resulting in successful intercourse was significantly improved with avanafil vs baseline ($P \leq 0.01$) for subjects with either type 1 or type 2 diabetes, all durations of diabetes history (< 2 to ≥ 10 years), and all ED severity subgroups. Additional analyses indicated that successful intercourse could be achieved in some subjects when initiated ≤ 15 minutes from dosing and, in other subjects, > 6 hours following dosing with avanafil. Most subjects attempted intercourse early; $> 60\%$ of sexual attempts were between 15 and 45 minutes after dosing. The most commonly reported adverse events (AEs) in the avanafil treatment groups were headache (7.8%), nasopharyngitis (3.1%), flushing (2.7%), and sinus congestion (1.9%). There were no drug-related serious AEs and no deaths reported during the study.

Conclusion: Avanafil was studied without food or alcohol restrictions and was found to be well tolerated and effective for mild-to-severe ED in men with type 1 or type 2 diabetes. Data also indicates that avanafil was effective as early as 15 minutes and in some subjects beyond 6 hours after dosing. As subjects with diabetes have a high incidence of ED, avanafil may provide an additional therapeutic option to benefit this population.

End of treatment mean and LS mean change from baseline to week 12 in coprimary end points (ITT)

	Placebo (n=127)		Avanafil 100 mg (n=126)		Avanafil 200 mg (n=126)	
	End of Treatment Mean	LS Mean Change from Baseline	End of Treatment Mean	LS Mean Change from Baseline	End of Treatment Mean	LS Mean Change from Baseline
Successful insertion (SEP2)	42.0%	7.5%	54.0%	21.5%*	63.5%	25.9*
Successful intercourse (SEP3)	20.5%	13.6%	34.4%	28.7%*	40.0%	34.0%*
IIEF-EF domain score†	13.2	1.8	15.8	4.5*	17.3	5.4*

* $P \leq 0.002$ vs placebo; †Baseline values for the IIEF-EF domain score were missing for 4 subjects (2 placebo, 1 avanafil 100 mg, and 1 avanafil 200 mg).

Clinical Trial Registration Number: NCT00809471

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The association between Neuropad testing with peripheral neuropathy in diabetes: a meta-analysis

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Background and aims: Published data suggest that the results obtained by visual test Neuropad correlate with presence and degree of peripheral neuropathy as well as with foot ulceration in subjects with diabetes. The aim of this meta-analysis was to examine the association between the results obtained by Neuropad testing with diabetic peripheral neuropathy and its performance in the diagnosis of this complication.

Materials and methods: A total of 8 published studies were identified involving 2146 patients (mean age 58.8 ± 11.2 years; mean duration of diabetes 13.1 ± 8.0 years) with type 1 and type 2 diabetes. A cut-off value of Neuropathy Disability Score ≥ 6 was used for the diagnosis of peripheral neuropathy. Neuropad response was evaluated as normal or abnormal according to complete colour change after 10 min of application. Weighted averages were reported as odds ratios (OR) with 95% confidence intervals using a fixed-effects model. Statistical heterogeneity scores were assessed using the Q and I^2 statistic.

Results: An abnormal Neuropad response was associated with increased OR [9.54 (7.10–12.83)] of peripheral neuropathy, with significant heterogeneity across the trials. An abnormal Neuropad response was associated significantly with peripheral neuropathy in all, but one, studies. The performance of Neuropad testing for the diagnosis of peripheral neuropathy was as follows: sensitivity: 0.85; specificity: 0.56, positive predictive value: 0.50, and negative predictive value: 0.86.

Conclusion: An abnormal Neuropad response after 10 min of application is associated with an almost 10-fold increased risk for peripheral neuropathy in subjects with diabetes. In addition, the test has a high sensitivity for the diagnosis of established peripheral neuropathy.

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Assessment of sudomotor function as a tool for cardiorespiratory fitness level evaluation: comparison with V02max

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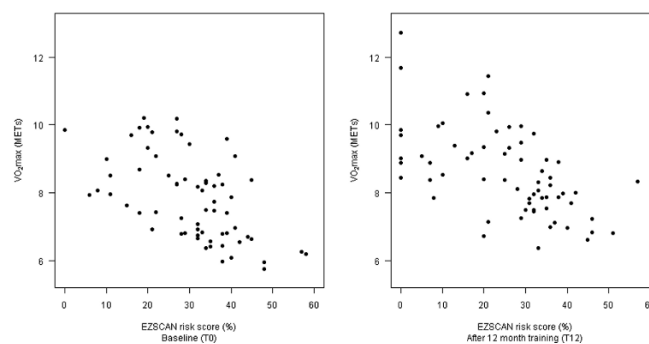
Background and aims: Physical inactivity is one of the main modifiable risk factors for cardiovascular (CV) and metabolic disorders. VO2max is the best method to assess cardio-respiratory fitness level but it is poorly accepted by the patient. Sudomotor dysfunction may develop early in cardiometabolic diseases. We compared the usual health analysis tests for CV risk evaluation to EZSCAN® a quick and non invasive method developed to assess sudomotor function.

Materials and methods: Cardio-respiratory fitness level was assessed in Finnish workers through a questionnaire, physical examination (BMI, blood pressure, waist, body composition) and VO2max using a maximal test on bicycle ergometer (T0). Based on local measurements of sudomotor function on hands and feet a cardiometabolic risk score was calculated. Sudomotor function was assessed before and after the exercise test to assess reproducibility of the method. In the subgroup of women with poor fitness level a training program was proposed. Cardio fitness level and sudomotor function were assessed after a 12 month follow-up (T12). Results are expressed as median (iqr). Correlation between V02max and EZSCAN® cardiometabolic risk score was performed using Spearman's rank correlation test.

Results: 537 women (age: 51 (13) years, BMI: 25 (6); VO2max: 8.9 (2.4) METs) and 113 men (age: 52 (11) years, BMI: 25 (4); VO2max: 12.2 (3.4) METs) were involved in the study. A difference in BMI, waist and body fat was present in men and women according to EZSCAN® risk score classification (no risk, moderate risk or high risk). For women and men the correlation between sudomotor function score and V02max was $r = -0.57$, $p < 0.0001$ and 0.48 , $p < 0.0001$ respectively. Coefficient of variation for sudomotor function measurements performed before exercise and after exercise was 5 %. In the subgroup of women with poor fitness level ($n = 65$) correlation between sudomotor function score and V02max was -0.58 , $p < 0.0001$ at T0 and -0.62 , $p < 0.0001$ at T12 (see Figure).

Conclusion: Sudomotor dysfunction as assessed by EZSCAN® is correlated to cardio-respiratory fitness levels and suggests that it can be used to assess

CV or metabolic disease risk and to follow individual preventive interventions.



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Sudomotor dysfunction and cardiovascular autonomic neuropathy assessed by spectral analysis of heart rate variability in diabetes

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Background and aims: Cardiovascular autonomic neuropathy (CAN) is a common but frequently overlooked complication of diabetes. Sweat glands are innervated by small unmyelinated sympathetic C-fibers that can be damaged very early in the disease course. A consensus statement of the American Diabetes Association has suggested that sudomotor function should be included in diagnostic test for early detection of diabetic neuropathy. SUDOSCAN a new quick, non invasive and quantitative method recently developed to simply assess sweat gland function was compared to spectral analysis of heart rate variability at rest and during exercise known to be valuable and reliable method for investigation of CAN.

Materials and methods: 241 patients with diabetes were involved in this study. The investigation consisted of spectral analysis of heart rate variability at rest (15 min) and during exercise (45 min, stair climbing) with measurement of average standard deviation of all NN intervals over 5 minutes (ASDNN-5min), High Frequency component (HF, linked to parasympathetic nervous system) and Low Frequency domain component (LF, linked to sympathetic nervous system). Sweat function was measured on hands and feet (areas with the highest sweat gland density). Patients placed their hands and feet on large electrodes where a low voltage ($<4V$) was applied during 2 minutes. Results are expressed as median (iqr), comparisons were done using Kruskal-Wallis test or quantile regression (for adjustment).

Results: As diabetic peripheral nerve function is disturbed in a length dependent manner, patients were classified according to the results of their feet sweat function expressed as the feet Electrochemical Sweat Conductance (ESC, μS): feet ESC $> 60\mu S$ no sweat dysfunction, $40-60\mu S$ moderate sweat dysfunction, $< 40\mu S$ high sweat dysfunction. Results of heart rate variability according to sweat dysfunction are displayed in the Table.

Conclusion: SUDOSCAN allowing quick and quantitative assessment of sudomotor function can be used for early screening of cardiovascular autonomic neuropathy in daily clinical practice before performance of more sophisticated, more specific but more time consuming tests.

Demography and heart rate variability data according to feet ESC						
	All (n=241)	Feet ESC > 60 μ S (n=117)	Feet ESC 40–60 μ S (n=63)	Feet ESC < 40 μ S (n=61)	p value Kruskall- Wallis test	p value adjusted on age and diabetes duration
Age (years)	53 (12)	51 (12)	53 (11)	57 (11)	<0.0001	-
Sex (men)	145	71	37	37	NS	-
Diabetes duration (years)	8 (11)	6 (9)	8 (10)	12 (11)	0.006	-
BMI (kg/m ²)	26.2 (4.9)	25.7 (4.3)	26.7 (4.9)	26.2 (5.5)	NS	-
HbA1C (%)	8.2 (2.4)	8.0 (2.1)	8.2 (2.7)	8.7 (2.6)	NS	-
ASDNN-5min at rest	30.0 (18.0)	31.9 (18.5)	29.1 (18.4)	25.3 (15.1)	0.024	NS
ASDNN-5min exercise	41.0 (20.0)	44.2 (25.3)	44.1 (23.2)	35.1 (12.4)	0.002	0.016
LF at rest(ms2)	220 (369)	248 (385)	220(278)	158 (187)	0.032	NS
LF exercise (ms2)	190 (324)	250 (397)	203 (277)	128 (161)	0.0003	0.035
HF at rest (ms2)	102 (185)	117 (188)	104 (158)	52 (155)	0.028	NS
HF exercise (ms2)	43 (82)	55 (117)	35 (78)	26 (48)	0.003	NS

PS 105 Somatic neuropathy and diabetic foot

1157

Oxidative stress and diabetic neuropathy

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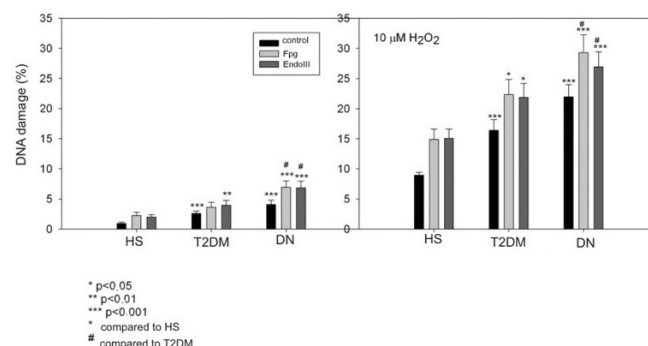
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Background and aims: Diabetic neuropathies (DN) are one of the most common complications of diabetes mellitus affecting ~ 50% of all diabetics. Experimental evidences suggest that hyperglycaemia-induced excessive reactive oxygen species (ROS) production and subsequent damage to proteins, lipids and DNA play a key role in degradation of neurons. The disturbances of antioxidant enzyme defense system (including superoxide dismutase - SOD, catalase - CAT and glutathione peroxidase - GPx) are also thought to contribute to the pathogenesis of DN. The study was designed to evaluate the level of oxidative stress parameters such as total antioxidants status (TAS), concentration of nitric oxide (NO), the level of DNA damage, and activity of antioxidant enzymes in type 2 diabetes mellitus (T2DM) patients with coexisting neuropathy.

Materials and methods: This study included 19 healthy subjects, 16 T2DM patients without and 16 T2DM patients with DN. The concentration of TAS and NO were measured in plasma. The level of DNA damage in lymphocytes of peripheral blood was assessed by the alkaline comet assay. The activity of SOD1, GPx and CAT were measured in erythrocyte hemolysate.

Results: No significant difference was observed between three groups regarding to age. Healthy subject revealed significantly higher NO plasma concentration ($8.9 \pm 1.6 \mu\text{mol/L}$) than T2DM patients with and without DN (7.6 ± 0.9 and 7.7 ± 1.3 , respectively) ($p < 0.05$). In T2DM patients with DN the level of TAS was lower than in patients without DNS and healthy subject (0.96 ± 0.3 vs 1.05 ± 0.21 vs 1.11 ± 0.3 mmol/L). Lymphocytes of T2DM patients with and without neuropathy exerted significantly higher level of endogenous and oxidative DNA damage compared to healthy subjects (Figure). Moreover, DNA of lymphocytes isolated from T2DM patients with and without DN was more susceptible for damage induced by hydrogen peroxide (Figure). The erythrocyte activity of SOD1 [U/gHb/100ml] was significantly lower ($p < 0.05$) in T2DM patients with DN (1895 ± 193) compared to healthy subject (2201 ± 529) and T2DM patients (2005 ± 320). The activity of GPx was markedly reduced ($p < 0.05$) in T2DM patients with DN (45.1 ± 8.8) compared to healthy subject (51.5 ± 9.2) and T2DM patients (50.8 ± 8.9). The CAT activity was lower in both groups of diabetics than in healthy subjects, but differences did not reach a significant statistical power.

Conclusion: We found the higher level of oxidative stress in T2DM people with DN. The higher level of oxidative stress was associated with lower antioxidant enzymes activity, depletion in plasma antioxidant status, reduced level of NO, and significantly higher level of DNA damage. These findings provide further evidence that the development of DN may be associated with inappropriate oxidant/antioxidant balance in people with diabetes mellitus.



Supported by: NN402 501 639

1158

The $\epsilon 4$ allele of APOE gene is associated with more severe diabetic peripheral neuropathy in type 2 diabetic patients

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Background and aims: The $\epsilon 4$ allele of APOE gene is a recognised risk factor for several neuromuscular diseases. However, some studies have cast doubt on this relation in diabetic neuropathy. Therefore, the aim of the present study was to investigate the potential adverse effect of $\epsilon 4$ allele of APOE gene on the severity of peripheral diabetic neuropathy in type 2 diabetic patients

Materials and methods: This study included 234 type 2 diabetic patients (120 men, age [mean \pm SD] 65.35 \pm 8.26 years, diabetes duration 12.08 \pm 8.01 years). Diabetic peripheral neuropathy was diagnosed and graded according to the Neuropathy Disability Score (NDS). Patients were grouped into those exhibiting mild (NDS lower than or equal to 6) and those exhibiting severe (NDS greater than 6) diabetic peripheral neuropathy. They were also genotyped for APOE gene and each NDS group was further divided into two subgroups, i.e. $\epsilon 4$ carriers and $\epsilon 4$ non-carriers. To evaluate the risk of severe diabetic peripheral neuropathy according to $\epsilon 4$ carrier status, logistic regression analysis was performed with adjustment for gender, age, duration of diabetes and HbA1c levels. Finally, the association between severity of neuropathy and the aforementioned patient characteristics was investigated as well.

Results: The relative risk of severe diabetic peripheral neuropathy was more than 5-fold increased in $\epsilon 4$ carriers (Adjusted OR: 5.26; 95% CI: 2.24–12.31; $p=0.0001$). Other significant risk factors were male gender ($p=0.036$), diabetes duration ($p=0.039$), and HbA1c levels ($p=0.020$).

Conclusion: Among type 2 diabetic patients, those carrying the $\epsilon 4$ allele of the APOE gene have a significantly higher risk of severe diabetic peripheral neuropathy.

1159

Evaluating a new device in the assessment of peripheral sensory neuropathy in diabetes: the Vibratip® study

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Background and aims: To evaluate the sensitivity, specificity, predictive values and likelihood ratios of a new device, Vibratip®, for detecting diabetic peripheral neuropathy (DPN) in routine clinical screening and to assess the intra-rater reliability of the Vibratip.

Materials and methods: The Vibratip was compared with the current reference for loss of protective sensation (Neurothesiometer®), as well as other bedside methods: 10g monofilament, 128Hz tuning fork and Neurotip®. 141 patients with type I or type II diabetes were studied. Sensation at five sites on each foot was assessed with a 10g monofilament, Neurotip and Vibratip and at two sites with a 128Hz tuning fork. A Neurothesiometer result at the hallux pulp $\geq 25V$ in either foot was considered indicative of DPN. Receiver operating characteristic (ROC) curves were produced and sensitivity, specificity, predictive values and likelihood ratios subsequently calculated. Sensation was assessed using the Vibratip twice in 18 patients and intra-rater reliability calculated using Cronbach's alpha.

Results: The sensitivities of the 10g monofilament, Vibratip, Neurotip and 128Hz tuning fork were found to be 84%, 79%, 74% and 69% respectively for the diagnosis of DPN. The negative likelihood ratios were 0.19, 0.25, 0.31 and 0.34, respectively. The ROC curves indicated that ≥ 2 insensate sites from 10 tested across two feet was predictive of a diagnosis of DPN for the 10g monofilament, Neurotip and Vibratip whereas ≥ 1 insensate site was predictive for the 128Hz tuning fork. The 10g monofilament had the best overall performance when compared with the Neurothesiometer, performing statistically significantly better than the 128Hz Tuning Fork and Neurotip ($p=0.0056$ and 0.0022 respectively). The alpha coefficient for Vibratip was 0.88, indicating good reliability.

Conclusion: In the detection of DPN as defined by the use of a Neurothesiometer, the performance of the 10g monofilament and Vibratip were comparable. Supported by: Samples of the Vibratip provided by the manufacturer.

1160

Skin autofluorescence relates to soluble receptor for advanced glycation endproducts in diabetic patients

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Background and aims: Advanced glycation endproducts (AGEs) play important role in the pathogenesis of diabetic complications. Their accumulation in the skin evokes elevation of skin autofluorescence (AF). According to novel findings, soluble receptors for AGEs (sRAGE) can reduce the pro-inflammatory effect of AGEs due to decreased activation of RAGE. The aim of this study was to evaluate skin autofluorescence levels in diabetic patients in respect of their sRAGE levels and other metabolic parameters.

Materials and methods: Skin AF was measured in 121 diabetic patients (62 Type 1 /T1DM/, 58 Type 2 /T2DM/; aged 54 \pm 16 yrs) and 26 healthy controls (aged 45 \pm 12 yrs) on forearm 3 times consecutively by AGE-Reader (Diagnoptics BV, Groningen, Netherlands). Results were expressed as arbitrary units (AU) and compared with age, diabetes duration, sRAGE concentration determined by ELISA kit, glycated hemoglobin HbA1c (expressed in IFCC units) and fructosamine. Albuminuria was expressed as albumine-creatinine ratio (ACR).

Results: Skin AF was significantly higher in both T1DM and T2DM as compared to healthy controls (2.35 \pm 0.56 AU, 2.62 \pm 0.69 AU vs 2.04 \pm 0.47 AU, ANOVA $p=0.0003$). Mean HbA1c was similar in both types of diabetic patients (7.5 \pm 1.8 % vs 7.0 \pm 2.1 %, NS). Significant relationship was found between AF and age (T1DM: $r=0.6$, $p<0.0001$, T2DM: $r=0.32$, $p<0.01$ and controls: $r=0.62$, $p<0.001$), and between AF and diabetes duration ($r=0.30$, $p<0.02$; $r=0.36$, $p<0.006$). However, no relationship was observed between AF and HbA1c or fructosamine in both T1DM and T2DM. Significant correlation was found between AF and sRAGE in all diabetic patients ($r=0.45$, $p<0.0001$), both in T1DM ($r=0.31$, $p<0.03$) and in T2DM ($r=0.63$, $p<0.0001$). Interestingly, patients with normal albuminuria (ACR<2.5 g/mol creatinine) had significantly lower AF in comparison to patients with positive (micro)albuminuria (T1DM: 2.25 \pm 0.52 vs 2.75 \pm 0.57 AU, $p=0.0003$; T2DM: 2.45 \pm 0.61 vs 3.01 \pm 0.84 AU, $p=0.0003$). Significant positive relationship was found between AF and (micro) albuminuria in both T1DM and T2DM ($r=0.36$, $p<0.005$ and $r=0.42$, $p<0.003$).

Conclusion: This is the first study describing relationship of the skin autofluorescence and serum sRAGE concentrations in Type 1 and Type 2 diabetic patients. In addition, higher levels of skin AF and sRAGE are related to more advanced vascular changes evaluated by albuminuria, but without any effect of actual diabetes control. It may be speculated that development of diabetic angiopathy is associated with markers of AGEs formation and in parallel with generation of protective mechanisms as free sRAGE molecules. Their role and interplay in the whole process of chronic diabetic complications will be confirmed in the follow-up study.

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1161

Diabetic foot ulcer risk stratification systems: Which one to choose?

A validation study

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Background and aims: Diabetic foot ulcer (DFU) risk stratification systems are a crucial tool for diabetic foot care, screening, resource allocation and complications' prevention. There are various systems with different characteristics and a recent systematic review has shown that little has been done for their validation and comparison. In fact, some of them were never externally validated nor their prognostic accuracy reported. Therefore, choosing which one to apply in our setting may not be straightforward. Thus, this study aims to validate and compare all the available systems [the University of Texas (UT), American Diabetes Association (ADA), International Working Group on the Diabetic Foot (IWGDF), Scottish Intercollegiate Grouping Network (SIGN) and Boyko and colleagues] for DFU occurrence prediction at 1 year (the lowest risk group recommended reevaluation).

Materials and methods: A retrospective cohort study was conducted on all patients with diabetes but without active DFU attending the CHVNG/Es-pinho EPE Diabetic Foot Clinic, from March 2006 to March 2011 ($n = 270$). Characterization variables (age, gender, diabetes' duration, type and treatment) and all those included in the stratification systems were assessed and registered at baseline [HbA1C, neuropathy and peripheral vascular disease, foot deformity, callus, visual and physical impairment, onychomycosis, tinea pedis, previous DFU and lower extremity amputation (LEA)]. Later, variables were collected from the patients' clinical files from mid to end of March 2011. All systems were applied at the data collection moment. Participants were followed for 1 year or until DFU occurred, i. e., a full-thickness skin defect distal to the malleoli requiring more than 14 days to heal. Variables' distribution was compared between the groups of patients with and without ulcers over the follow-up course, using the appropriate statistical tests (according to the variables' type and normality of distribution). Significance was defined as $p < 0.05$.

Results: Type 2 diabetes was present in 99.6% of patients, 44.2% were men and (at baseline) the median age was 66 years and diabetes duration 16 years. Median follow-up was 12 months (range 1–12) during which 29 participants (10.7%) developed a DFU. Variables associated with DFU occurrence at 1 year were age, HbA1C, Semmes-Weinstein monofilament and/or tuning fork sensitivity alteration, number of pulses present, previous DFU or LEA. For all the systems there were more ulcerations as the risk group increased (χ^2 for association and trend $p < 0.001$). The UT system presented an area under the receiver operating curve (AUC) of 0.70 [95% confidence interval (CI) 0.58–0.81], the ADA system of 0.82 (95% CI 0.77–0.88), the IWGDF system of 0.84 (95% CI 0.77–0.91), the SIGN system of 0.75 (95% CI 0.68–0.82) and the Boyko and colleagues system of 0.82 (95% CI 0.74–0.90).

Conclusion: There are several risk stratification systems that can be used in clinical practice to detect those at higher risk of DFU occurrence and they seem to be equally accurate. Although the selection of which system to apply is still unclear, this study shows that all of these systems present a high accuracy and therefore are a valuable tool. Nevertheless, further validation studies should be performed in larger samples and in different settings. There is also a great need to assess the reliability of the systems and their components.

1162

Endothelial dysfunction in diabetic subjects with neuropathic foot ulceration

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Background and aims: Diabetic patients with foot ulceration have increased risk of cardiovascular disease (CVD) that may seem logical for patients with ischemic ulcers. However, increased CVD had also been reported in patients with neuropathic foot ulceration (NFU) without clear explanation. Current evidence suggests that endothelial dysfunction contributes to the formation, progression, and complications of atherosclerosis. The aim of this study was to evaluate endothelial dysfunction as a possible element for excess CVD burden in diabetic patients with NFU.

Materials and methods: The study included forty diabetic subjects with NFU (The Neuropathic Group) and twenty diabetic subjects without peripheral nerve dysfunction (The Diabetic Group). The two groups were carefully selected to be matched for age (54.3 ± 7.6 vs. 56.3 ± 7.5 yrs, $P = 0.337$), sex (M/F: 21/19 vs. 12/8, $P = 0.784$), BMI (39.5 ± 7.1 vs. 36.8 ± 5.2 , $P = 0.103$), systolic and diastolic blood pressure (147.5 ± 21.9 vs. 140.8 ± 18.2 , $P = 0.213$ and 90 ± 9.9 vs. 88.3 ± 9.2 , $P = 0.503$ mmHg respectively). All subjects were treated with insulin. Subjects with renal impairment, peripheral arterial disease, smoking or using oral hypoglycemic or lipid lowering drugs were excluded. Endothelial function was evaluated by flow-mediated dilation (FMD) in the brachial artery after reactive hyperemia using high-resolution ultrasound (Toshiba Powervision 6000 with a 7.5 MHz linear array transducer). Carotid intimal medial thickness (CIMT) and FMD were evaluated by a single radiologist blinded to the study variables.

Results: FMD in the brachial artery was much reduced in the neuropathic group in comparison with the diabetic group (4.5 ± 4.4 vs. 16.1 ± 8.5 %, $P = 0.001$). Both groups were comparable in CIMT (1.2 ± 0.4 vs. 1.3 ± 0.4 mm, $P = 0.318$), A1c% (8.6 ± 0.7 vs. 8.8 ± 0.8 , $p = 0.413$), total cholesterol (205.6 ± 12.7 vs. 203.3 ± 16.5 mg/dl, $P = 0.575$) and triglycerides (201.5 ± 30.8 vs. 211.5 ± 36.3 mg/dl, $P = 0.299$). However, diabetes duration and waist circumference were significantly increased in the neuropathic group (17.3 ± 6.9 vs. 8 ± 5.8 yrs, $p =$

0.000 and 125.6 ± 9.7 vs. 118.3 ± 13.1 cm, $P = 0.022$ respectively). FMD correlated inversely with CIMT ($r = -0.319$, $P = 0.045$), age ($r = -0.34$, $P = 0.032$) and ulcer area ($r = -0.453$, $P = 0.005$) but not with duration of diabetes, BMI, waist circumference, ulcer duration, systolic or diastolic blood pressure. FMD was much reduced in men in comparison to women (2.9 ± 4.7 vs. 6.1 ± 3.4 %, $P = 0.023$).

Conclusion: The results of this study demonstrate more remarkable endothelial dysfunction in diabetic subjects with NFU in comparison to a matched group without peripheral nerve dysfunction. Endothelial dysfunction was greater in men and FMD was inversely and strongly related to CIMT, age of the patients and ulcer area. We suggest that attention should be paid to endothelial dysfunction in diabetic subjects with NFU in order to save not only limbs but also lives.

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Are certain psychological parameters independent risk factors for outcomes of diabetic foot therapy?

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Background and aims: The healing of the diabetic foot (DF) could depend not only on complex therapy served by health providers but also on patient compliance that is relying on certain psychological parameters of treated patients. The aim of our study was to assess possible associations of selected psychological parameters with the outcomes of DF therapy.

Materials and methods: 59 patients with DF (mean age 58.9 ± 9.5 years, diabetes duration 20.1 ± 11.4 years, HbA1c according to IFCC $6.3 \pm 2\%$) whose were consecutively treated in foot clinic from 1/2010 to 3/2010 were included into our study. In these patients certain psychological parameters were evaluated by following psychological tests - degree of pain by Visual Analog Scale, degree of depression by Geriatric depression scale, QoL by 4 domains (Physical, Psychological domains, Social Relationships and Environment) based on WHOQoL-Bref test, consequences of stress action by Social Readaptation Scale and discrimination of stress factors by Stress Scale. The follow up of one year of the outcomes of DF therapy was performed after the inclusion into our study. The primary endpoints were defined by Global Impression-Improvement Score as the improvement of DF (1–3; including healing and improvement of local findings) and the worsening of DF (5–7; including progression of local findings, lower limb amputations and death). During the observed period 22/59 study patients healed (37.2%), 15/59 underwent amputations (25.4%) and only 1/59 death was recorded (1.7%). The possible associations of psychological parameters with secondary end-points (number of hospitalizations, new ulceration development) were also assessed.

Results: Neither QoL (Physical domain - 12.2 ± 2.8 vs. 12.3 ± 3.1 , Psychological domain - 14.4 ± 2.4 vs. 14 ± 3.1 , domain of Social Relationships 14.7 ± 2.8 vs. 14 ± 3.3 and of Environment 13.8 ± 2.1 vs. 13.7 ± 2.5 ; NS) nor other psychological parameters including the degree of depression (12.4 ± 3.6 in patients with the improvement of DF vs. 12.9 ± 2.6 in patients with the worsening of DF; NS) were significantly associated with the primary end-points of DF therapy. Similarly secondary end-points were not significantly influenced by selected psychological parameters.

Conclusion: Selected psychological parameters had no predicting values for 1-year follow up therapy outcomes in patients with the DF. Possibly other redefinition (healed x unhealed DF) of primary end-points will be useful for statistical analyzing due to possible overlapping of patients psychological characteristics related to outcomes of DF therapy.

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1164

Risk predictors of lower-limb amputation in patients with type 2 diabetes mellitus in the fenofibrate intervention and event lowering in diabetes study

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Background and aims: Lower limb amputations associated with type 2 diabetes cause major disability and portend a high mortality over the next few

years. The aim of this analysis was to identify important risk predictors for future lower limb amputation among a large cohort of 9795 patients with type 2 diabetes mellitus in the Fenofibrate and Event Lowering in Diabetes (FIELD) study.

Materials and methods: Patients were randomised to receive co-micronised fenofibrate 200 mg/day or matching placebo over 5 years, and amputation events (a prespecified tertiary endpoint) were documented at 6-monthly intervals. Time to amputation was evaluated in multivariable proportional-hazards regression analysis using exhaustive-search methods to develop predictive models.

Results: The main predictors of the first on-study amputation were a history of previous diabetic skin ulcer or non-traumatic amputation (hazard ratio [HR] 5.6), neuropathy (HR 3.0), peripheral vascular disease (HR 2.6), age over 65 years (HR 2.04) and height (HR 1.5 per 10 cm taller) (all $P < 0.001$). Other significant predictors included smoking, albuminuria, HbA1c, retinopathy and PTCA. Increasing risk of cardiovascular disease events and death was associated with increasing amputation risk (trend $P < 0.0001$).

Conclusions: Classical markers of macrovascular and microvascular risk predicted amputations. We also identified height as a major predictor of diabetic amputations, independent of the presence of neuropathy, confirming a previous report from an observational study. These findings could enable more aggressive targeting of modifiable risk factors among patients at high risk of amputations and subsequent cardiovascular events who would benefit most from aggressive therapeutic intervention.

Clinical Trial Registration Number: ISRCTN64783481

Supported by: Abbott Laboratories

1165

Charcot neuroarthropathy is a risk factor of coronary stenosis independent of calcium score

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Background and aims: Type 2 diabetic subjects have an increased cardiovascular mortality. This elevated risk is linked to an accelerated vascular atherosclerosis, in particular coronary atherosclerosis. Coronary artery calcium (CAC) is an integral part of atherosclerotic coronary heart disease and there are several studies supporting the role of CAC score for prediction of myocardial infarction and cardiovascular mortality. The aim of our small cross-sectional study was to explore the differences in coronary calcium score (CCS, a marker of CAC) in four different populations.

Materials and methods: 10 health subjects, 10 uncomplicated type 2 diabetic patients, 10 type 2 diabetic patients with autonomic neuropathy and 10 type 2 diabetic subjects with Charcot Osteoarthropathy. The diagnosis of autonomic neuropathy was made with cardiovascular tests while Charcot foot was defined according to clinical signs and symptoms. CCS and CT Coronary Angiography were both performed by a 64-row multidetector CT scanner with ECG-gating.

Results: The three diabetic groups did not present significant differences about the variables known to influence coronary atherosclerosis (age, sex, disease duration, lipids, HbA1c, Waist-to-Hip ratio). Comparing diabetics with health controls, only HbA1c was significantly different ($p < 0.001$). All Charcot and diabetic patients with autonomic neuropathy had a cardiovascular autonomic score > 5 compared to controls and uncomplicated diabetics (all < 2 ; $p < 0.001$). With regard to total CCS, it was significantly higher in diabetic patients compared to controls (179 AS [112–862] vs 3.5 AS [3–4], respectively; $p = 0.009$), without differences among the three diabetic groups. Notwithstanding this, the rate of Charcot patients with coronary stenosis $> 50\%$ (clinically significant stenosis) was higher compared to no-Charcot patients (79 vs 52%; $p = 0.038$).

Conclusion: In conclusion, our study supports previous findings about CCS of diabetic subjects and underlines as Charcot Osteoarthropathy could be considered a prognostic marker of a more severe coronary atherosclerotic lesions. Further, larger and hard-points studies are necessary to confirm these data.

PS 106 Wound healing: experimental

1166

Insulin signalling regulates keratin-intermediate filaments structure and function

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Background and aims: In recent years, new roles for insulin have emerged, including regulation of cellular proliferation, differentiation, and apoptosis. However, it has not yet been determined whether these newly recognized insulin regulatory functions have any clinical significance. Previously, we demonstrated that insulin is essential for skin proliferation and differentiation. Furthermore, we have found that insulin receptor (IR) deficient mice have impaired wound healing. This skin pathology was associated with abnormal structure of epidermal keratin 1 (K1) filaments. K1 is a member of the intermediate filaments family. These cytoskeletal proteins give the cell its strength, shape and motility. Thus, insulin-induced changes in the structure and assembly of keratins might lead to impaired cellular function. To further investigate insulin regulation of K1 structure and assembly, we further studied skin cells isolated from IR and IRS1 knockout mice (IRKO and IRS1KO respectively). In addition, we have established a human based cell model in which IR or IRS1 were inactivated.

Materials and methods: Primary cultures of keratinocytes are isolated from newborn IRKO, IRS1KO and control mice as previously set in our lab. IR or IRS1 silencing in human skin cells was achieved by expression of IR or IRS1 targeted shRNA in human keratinocyte HaCaT cells (IRKD and IRS1KD respectively). K1 assembly, structure and function are evaluated by Western Blot analysis, indirect immunofluorescence and confocal microscopy.

Results: Lack of IR resulted in a marked decrease in insoluble K1 filament level, indicating that K1 assembly is impaired in the IRKO cells. This decrease was associated with abnormal structure of K1 filaments, as visualized by indirect immunofluorescence (fig. 1). Interestingly, we have found that these changes were associated with changes in K1 serine phosphorylation. While in control cells insulin increased K1 phosphorylation, there was no K1 phosphorylation in the IRKO cells. Lack of IRS1 resulted in further deterioration in K1 expression, as IRS1KO murine cells show a decrease in both soluble and insoluble K1 expression. Thus, lack of IRS1 affects both the assembly as well as the expression of K1. These results were further corroborated in the human IRKD and IRS1KD cells. Human IRKD cells show a decrease in insoluble K1, whereas IRS1KD cells show a decrease in insoluble and soluble K1, as was found in the murine cells. Interestingly, both IRKD and IRS1KD cells had significantly lower proliferation rates compared to control keratinocytes. In addition, IRKD cells demonstrated decreased adhesion to ECM proteins.

Conclusion: Our results demonstrate for the first time that insulin regulates the structure, phosphorylation and function of keratin intermediate filaments. These findings suggest that insulin signaling is essential for normal skin development, and that impaired insulin signaling, as occurs in diabetes, directly contributes to the pathogenesis of impaired wound healing in diabetes.

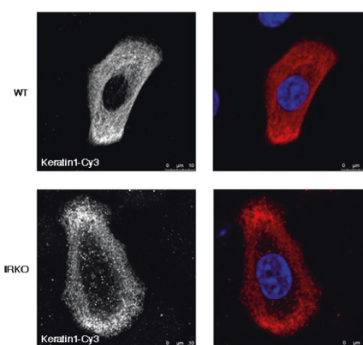


Figure 1: Indirect immunofluorescence of keratin 1 filaments. Primary IRKO and WT murine keratinocytes were grown on coverslips. K1 protein was visualized by indirect immunofluorescence using a rabbit anti-mouse keratin 1 antibody as the primary antibody. The secondary antibody was Cy3-labeled goat anti-rabbit IgG (red). The nuclei of the cells were visualized by DAPI staining. Fluorescence signals were analyzed by confocal microscopy ($\times 63$).

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Impaired mast cell function affects wound healing in diabetesA. Tellechea^{1,2}, J.M. Zabolotny¹, L. Pradhan¹, E. Leal², A. Kafanas¹, S. Kuchibhotla¹, E. Carvalho^{2,1}, A. Veves¹;¹Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, USA, ²Center for Neurosciences and Cell Biology, University of Coimbra, Portugal.

Background and aims: Recent studies suggest that neuropeptides and mast cells participate in wound healing but the mechanisms of their action are not clear. Our hypothesis is that skin mast cells are dysfunctional in diabetes due to neuropeptide deficiency, contributing to impaired wound healing.

Materials and methods: We assessed wound healing in both streptozotocin-induced diabetic (STZ-DM) and non-diabetic (non-DM) mast cell deficient mice (KitW/KitW-v) and their wild type (WT) littermates. Two 6-mm excision wounds per mouse were created on the dorsum of the anesthetized mice, wound healing was followed for ten days and wounds were harvested afterwards. In some animals, one wound was treated with Substance P (SP) and one with placebo. Skin O₂ hemoglobin saturation was determined prior to wounding (day 0) and at day 10 using hyperspectral imaging. To confirm the presence/absence of mast cells and to evaluate gene expression of growth factor and cytokines at the skin level, toluidine blue staining and q-RT-PCR were performed.

Results: Wound healing was delayed in mast cell deficient mice compared to WT mice. This impairment was evident at days 5 to 10 in the non-DM group and at days 8 to 10 in the STZ-DM group ($p < 0.05$). Furthermore, hemoglobin saturation was significantly lower in both non-DM and STZ-DM mast cell deficient mice when compared to WT non-DM and STZ-DM mice, respectively, at day 10 post-wounding ($p < 0.01$). Topical treatment of the wounds with SP accelerated wound closure in both non-DM and STZ-DM WT mice ($p < 0.05$), but had no effect in the KitW/KitW-v mice. As expected, the KitW/KitW-v mice did not have any mast cells at the skin level. WT STZ-DM mice had a lower mast cell count when compared to the non-DM mice but showed higher mast cell degranulation levels ($p < 0.05$). At day 10 post-wounding, gene expression of FGF, EGF, VEGF and MPO was decreased while gene expression of KC, IL-6, MMP-9 and SP was increased in the skin of KitW/KitW-v mice when compared to WT ($p < 0.05$).

Conclusion: Mast cell deficiency severely impairs wound healing in both DM and non-DM settings and leads to skin hypoxia and aberrant expression of growth factor and cytokines. STZ-induced diabetes affects skin mast cell abundance and function. SP exerts its beneficial effect on wound healing, at least partly, through mast cell function.

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Protein tyrosine phosphatase 1B (PTP1B) deficiency promotes wound healingJ.M. Zabolotny¹, A. Tellechea^{1,2}, E. Leal², I. Kontoes², S. Kuchibhotla², L. Pradhan², E. Carvalho³, A. Veves²;¹Division of Endocrinology, Diabetes, and Metabolism, Beth Israel Deaconess Medical Center, ²Surgery, Beth Israel Deaconess Medical Center, Boston, USA, ³Center for Neurosciences and Cell Biology, University of Coimbra, Portugal.

Background and aims: The major complication of diabetes leading to hospitalization is chronic diabetic foot ulceration. Growth factors play a key role in wound healing, and wound site growth factor deficiency is associated with impaired healing of diabetic foot ulcers. PTP1B negatively regulates a network of growth factor and cytokine signaling pathways that promote wound healing, including vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), and leptin via dephosphorylation of their respective receptors or associated kinases. In mice, PTP1B expression is increased in a variety of tissues by obesity- or diabetes-associated inflammation and is elevated in response to tumor necrosis factor alpha (TNF α) treatment. The aim of the current studies was to determine whether PTP1B affects skin wound healing progress in healthy mice as well as in the context of diabetes.

Materials and methods: Wound closure kinetics of full thickness excisional skin wounds in PTP1B-deficient mice were compared to those of wildtype control mice. The effect of PTP1B deficiency on wound healing was also compared that of topically applied leptin, which is known to promote wound

healing. Medical hyperspectral imaging (MHSI), an advanced form of spectroscopy that provides a 2D map of tissue oxygenation, was used to measure tissue oxyhemoglobin and deoxyhemoglobin of the periwound area pre- and post-wounding to determine oxygen saturation of hemoglobin during the healing process. Mice were also made diabetic by streptozotocin treatment and wound closure kinetics examined.

Results: PTP1B deficiency in mice enhanced wound closure by ~25% at day 3 and by ~50% at day 10 post wounding compared to wildtype control mice ($p \leq 0.05$ for both measurements). Importantly, PTP1B deficiency also enhanced the ability of topical leptin to promote wound healing compared to that observed in wildtype mice receiving topical leptin. Prior to wounding, oxygen saturation of hemoglobin measured by MHSI in skin of wildtype and PTP1B-deficient mice was similar. In contrast, periwound hemoglobin oxygen saturation was enhanced by ~10% at day 3 and by ~20% at day 10 post wounding in PTP1B-deficient mice compared to wildtype, consistent with experimental and clinical findings linking tissue oxygen saturation with increased rates of wound healing. The effects of PTP1B deficiency on wound healing were similar in diabetic mice. Wound healing was enhanced by ~50% in diabetic PTP1B-deficient mice compared to diabetic wildtype controls by day 10 post wounding ($p \leq 0.05$).

Conclusion: These data show that augmenting signal transduction via PTP1B deficiency enhances skin wound healing in mice in both the presence and absence of diabetes and importantly, increases the efficacy of topically applied growth factors to promote healing. These results suggest that PTP1B inhibition may have utility treating chronic foot ulcers in diabetes.

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Mesenchymal stem cells improve impaired foot ulcer healing in diabetic rats

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Background and aims: Diabetes mellitus is one of the main causes of non-traumatic amputation of lower extremities, which substantially impair quality of life. Therefore, establishment of the treatment of foot ulcer in diabetic patients is required. Several therapies such as antiplatelet agents, prostaglandin analogues and/or local administration of bFGF have clinically been available as noninvasive treatment. Moreover, beneficial effects of mononuclear cell transplantation on foot ulcer have also been reported. It has been reported that mesenchymal stem cells (MSCs) produce and secrete various cytokines including VEGF and bFGF, which suggest that transplantation of MSCs would be useful for treatment of foot ulcer. Here we evaluated the therapeutic effects of MSC transplantation on impaired foot ulcer healing in the diabetic condition using a diabetic foot ulcer model and human keratinocytes (HKCs) primary cultured under the high glucose condition.

Materials and methods: 1) Isolation of MSCs: MSCs were isolated from bone marrow of 5 week-old SD rats. The characters and various growth factor producing abilities of MSCs were evaluated by FACS and RT-PCR, respectively. 2) HKC culture: HKCs were cultured under the 6 mM (NG) or 12 mM (HG) glucose condition with or without MSC-conditioned media (MSC-CM) for 72 hours. Cell viability was evaluated by MTS assay. 3) Diabetic foot ulcer model: Diabetes was induced by intraperitoneal injection of Streptozotocin to 6 week-old SD rats. After 8 weeks of diabetes, a full thickness 4mm diameter punch hole were created in the hind paw of normal (N) and diabetic rats (D). At the same time, 1×10^6 MSCs labeled with PKH were injected around the wounds. Wounds were photographed and the sizes of the remaining wounds were measured every other day. Blood flow on the wound beds (BF) was measured by laser Doppler flowmetry. Wounds samples were excised and histological evaluation was performed.

Results: 1) MSCs were positive for CD29 and CD90 and negative for CD34 and CD45. mRNA expressions of VEGF, bFGF, NGF, EGF and KGF were detected in MSCs. 2) HKC viabilities were significantly decreased under the HG condition (NG: $100 \pm 4\%$, HG: $87 \pm 4\%$ ($p = 0.02$)). MSC-CM significantly restored the impaired HKC viabilities under the HG condition (HG with MSC-CM: $192 \pm 21\%$ ($p < 0.01$ vs HG without MSC-CM)). 3) D showed significant hyperglycemia (N: 6.8 ± 0.2 mM, D: 24.1 ± 2.2 mM) and MSC transplantation did not affect glycemic levels. MSCs labeled with PKH were survived in dermal and subcutaneous tissues even three weeks after transplantation. At day 6, the percentage of wound area to original wound area was $62 \pm 1\%$ in N without MSC treatment (NC), $60 \pm 5\%$ in N with MSC treatment (NT), $76 \pm 4\%$ in

D without MSC treatment (DC) ($p=0.02$ vs NC) and $60\pm 2\%$ in D with MSC treatment (DT) ($p=0.02$ vs DC). Complete wound healing was observed at day 16.5 ± 1.8 in NC, 14.0 ± 1.2 in NT, 21.5 ± 1.1 in DC ($p=0.04$ vs NC) and 16.8 ± 0.7 in DT ($p=0.02$ vs DC). Decreased BF in DC was improved by MSC transplantation. Histological evaluation revealed the delayed wound reepithelialization in DC, which was ameliorated by MSC treatment.

Conclusion: These observations indicate that MSC transplantation would be useful for treatment of diabetic foot ulcer through not only ameliorating impaired microcirculation but also enhancing reepithelialization by keratinocytes.

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Blocking notch pathway improves wound healing in diabetic mice

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Background and aims: Wound healing is a complex process that needs coordination of several mechanisms such as cell proliferation, cell differentiation and angiogenesis. Diabetes has a repressive effect on wound healing and it is a high need for a better understanding of the pathophysiological pathways behind it in order to design new therapeutical approaches. The Notch system consists of several receptors (Notch 1-4) and ligands with a high specific cell dependent effect. The Notch signaling is activated after interaction between a ligand and one of the receptors. This interaction is transmitted intracellularly by a process involving proteolytic cleavage of the receptor by gamma-secretase complex. This results in the release of the intracellular domain of the Notch receptor (NICD) which translocates to the nucleus and activates several target genes. We have studied the modulation of the Notch pathway in diabetes having in mind the essential role played by the Notch system in the regulation of cell differentiation and angiogenesis.

Materials and methods: The effect of hyperglycemia on Notch system was studied *in vitro* in human dermal fibroblasts (HDF), mouse embryonic fibroblasts or in different animal models of diabetes (db/db mice and Goto-Kakizaki (GK) rat) using the appropriate method (western blot, Notch-responsive luciferase reporter gene assay or evaluation of target genes by quantitative RT-PCR). The functional consequences of the Notch system modulation were studied *in vitro* by the assessment of the migration of HDFs and by angiogenesis assay (using human endothelial cells). Notch pathway inhibition was induced either chemically with gamma-secretase inhibitors, (DAPT, L-685,458) or by specific siRNA silencing of the Notch receptors. The effect of the notch inhibition on wound healing was evaluated in db/db mice. Dermal punch biopsies were performed on the back of the animals and the wound healing rate was followed by digital photography. The angiogenesis was evaluated by lectin staining, the granulation tissue by hematoxylin & eosin staining and recruitment of endothelial precursors cells (EPCs) by QRT-PCR.

Results: Hyperglycemia activates Notch pathway at several levels as shown by increased NICD level, increased reporter gene activity and enhanced expression of several essential target genes as evaluated both *in vitro* and *in vivo*. The inhibitory effect of high glucose on migration of HDF and angiogenesis was cancelled by blocking the notch signaling with different gamma-secretase inhibitors (DAPT or L-685,458). Specific inhibition of different Notch receptors (1-4) by siRNA pointed out to a crucial role of Notch1 in migration and angiogenesis. Local treatment with gamma-secretase inhibitors (DAPT or L-685,458) improved the wound healing in db/db mice (Percentage of wound closure on day 12 was $60\pm 2\%$ (DAPT), $70\pm 4\%$ (L-685,458) and $43\pm 5\%$ (Placebo) respectively ($p<0.01$). Blocking Notch pathway in diabetic wounds was followed by increase in granulation and epidermal formation, increase in blood vessel formation and increase in expression of chemokine (SDF-1 alpha) responsible for better recruitment of EPCs.

Conclusion: Notch signaling was activated by hyperglycemia with deleterious effects on cell migration and angiogenesis. Blocking the overactive notch pathway by local treatment with gamma-secretase inhibitors improved wound healing rate in diabetic animal model.

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Neurotensin and chitosan-based dressings: a new approach for diabetic wound healing treatment

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Background and aims: Diabetes mellitus is one of the most widespread diseases in the world. It may cause chronic and non-healing diabetic foot ulcers (DFU) which decrease the welfare of patients. Recent studies indicated that some neuropeptides like Substance P, NPY, and CRH may act as inflammatory modulators and may improve the wound healing process. Natural biopolymers like chitosan, collagen and their derivatives, are presently receiving attention as wound dressing materials for wound healing applications. This is mostly due to some of their several favorable properties such as biocompatibility, biodegradability, non-toxicity and favorable biological behavior. Some chitosan derivatives such as N-carboxymethyl chitosan (CMC), N-carboxybutyl chitosan (CBC) and N-succinyl chitosan (SC) are known to be potential materials for wound healing applications. Employing these chitosan derivatives simultaneously as dressings and as platforms for the delivery of neuropeptides, such as, neurotensin (NT) has not yet been evaluated. This has been addressed in this work.

Materials and methods: Synthesis of CMC, CBC and SC: Chitosan reacted with glyoxylic acid, levulinic acid or succinic anhydride, for the synthesis of CMC, CBC and SC, respectively. *In vitro* release kinetics: Known amounts of a GSH solution (5mM) were loaded into previously weighted samples of each polymer. After drying, samples were immersed in PBS at different pHs at 37°C and for 8h. The quantification of released glutathione was based on the Ellman's Test. Release results with GSH were considered to choose polymeric samples for the Animal Model experiments. Animal model: Diabetes was induced by an intraperitoneal injection of 200mg/kg streptozotocin (STZ) dissolved in 200ml citrate buffer (pH 4.2) or buffer alone (non-diabetic mice). Control or STZ-treated mice were anesthetized and two 6 mm excision wounds, 2 cm apart, were created dorsally using a punch biopsy tool. CBC alone, NT alone (50ug/wound/per day), CBC loaded with NT (50ug/wound/per day) or PBS were placed daily on wounds and the progress of wound closure was monitored by acetate tracing up to 10 days.

Results: Release kinetics experiments showed that GSH release was not strongly affected by the release media pH (over a range between 6 and 8). However, SC presented a faster GSH release when compared with CMC and CBC at pHs of 6 and 7. In *in vivo* studies, CBC treated wounds showed a significant reduction in the wound area as compared to PBS treated wounds (29.5 %: $p<0.001$), wounds treated with NT alone also showed a reduction of (7.4%: $p<0.02$), while it is with the combination of the two treatments that we observed the greatest reduction in wound area (32.8%, $p<0.001$), already at 3 days post-wounding, in normal mice while in diabetic animals the observed differences are even more pronounced with the combined treatments showing a reduction of 39.8%, $p<0.001$. In addition, wounds treated with CBC and CBC plus NT, at day 10 in the same experiments, showed a smaller difference in wound reduction as compared to the PBS treated wounds at day 10.

Conclusion: Results demonstrated that CBC films incorporated with NT could be potentially advantageous as NT-releasing wound dressings for the treatment of diabetic foot ulcers.

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Neurotensin decreases TNF alpha-induced cell death in keratinocytes and macrophages: possible important role in diabetic wound healing

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Background and aims: Neuropeptides are known to be important in wound healing, particularly in diabetic wound healing. Although the role of neurotensin in wound healing is not well known. Moreover, a chronic inflammatory state in diabetes is responsible for the impairment of wound healing and tumor necrosis factor-alpha (TNF-alpha) appears to have an important role. In this study we aim to investigate the effects of neurotensin in TNF-alpha-induced changes in skin cells, keratinocytes and macrophages.

Materials and methods: Keratinocyte (HaCat) and macrophage (Raw 264.7) cell lines were incubated with 10ng/ml of TNF-alpha and/or 100 nM of neurotensin for 6 and 24h. We evaluated cell viability with the MTT assay and cell

death with nuclear staining (DAPI). Caspase-3, p38 MAPK, NF κ B activation and the detection of oxidized carbonyl groups were evaluated by western blot. Nitrosative stress was detected with nitrotyrosine staining.

Results: We found that 24h of incubation with TNF- α decreased HaCat and Raw cell viability ($78.7 \pm 4.0\%$ and $82.8 \pm 4.6\%$ of control, $p < 0.05$, respectively) and also caused cell death ($6.2 \pm 0.8\%$ and $4.3 \pm 0.3\%$ of total cells, $p < 0.05$, HaCat and Raw cells respectively). These effects were reverted by neurotensin. The active form of caspase-3 was found to be significantly increased with TNF- α in both cell types (HaCat, $p < 0.05$, $369 \pm 84.4\%$ of control; raw, $p < 0.01$, $329.2 \pm 50.4\%$ of control) and neurotensin decreased this activation suggesting that caspase-3 is involved in cell death. Neurotensin decreased TNF- α -induced activation of the p38 MAPK and NF κ B pathways. In addition, neurotensin decreased TNF- α -induced oxidative and nitrosative stress in skin cells.

Conclusion: Neurotensin inhibits the TNF- α -induced decrease in skin cell viability and cell death. The protective effects of neurotensin appear to be mediated by a decrease in oxidative and nitrosative stress, and by the inhibition of caspase-3, p38 MAPK and the NF- κ B pathways. These results suggest that neurotensin can have a protective role in chronic inflammatory conditions, particularly in chronic diabetic wounds.

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Effects of Astragalus polysaccharides on the expression of MMP-2 and MMP-9 in human dermal fibroblasts derived from diabetic foot ulcers

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Background and aims: The studies on the root of membranous milk vetch have showed that it can drain abscess out, promote the growth of cells and tissues, and recovered the function of dying cells. Astragalus Polysaccharides derived from the root of membranous milk vetch (*Astragalus membranaceus*) is still unclear for healing chronic diabetic foot ulcers whether the changes of diabetic ulcer fibroblasts can be reversed, whether the concentrations of MMPs can be reduced. We inquired into the effects of Astragalus Polysaccharides (AP) on the proliferation of fibroblasts (Fb), the activity and expression of MMP-2 and MMP-9 induced by IL-1 β in Fibroblasts from diabetic foot ulcers (DFU). The study was approved by the Hospital Ethics Committee.

Materials and methods: On the basis of analyzing the difference of IL-1 β between acute traumatic lesions in 9 patients with diabetes mellitus (DM) and 12 patients with DFU, The Fibroblasts proliferation, the activity and the expression of MMP-2, MMP-9 were tested After being incubated with different concentration of IL-1 β and AP.

Results: The concentration of IL-1 β in fluid from DFU was higher significantly than that of diabetic traumatic wounds ($p < 0.001$). Compared with the control group, 0.5–5ng/ml IL-1 β significantly stimulated fibroblasts proliferation, 50ng/ml IL-1 β were of no effect, 500ng/ml IL-1 β could inhibit fibroblasts proliferation. 50ng/ml IL-1 β +AP100 μ g/ml and 50 ng/ml IL-1 β +AP500 μ g/ml had positive effects on the fibroblast proliferation ($P < 0.001$). The activity of MMP-2, MMP-9 in 100 μ g/ml AP were lower significantly ($P = 0.006$) while the activity of MMP-2, MMP-9 in 50ng/ml IL-1 β were significantly higher than those in control group ($P = 0.000$). The level of MMP-2, MMP-9 protein expression in 100 μ g/ml AP was significantly lower ($P = 0.002$) and the level of MMP-2, MMP-9 protein expression in 50ng/ml IL-1 β was significantly higher than those in control group ($P = 0.0001$).

Conclusion: The AP not only promote the fibroblast proliferation, but also inhibit the activity and protein expression of MMP-2, MMP-9, might promote diabetic foot ulcers healing.

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Efficacy of a two-step screening strategy for gestational diabetes in an area with a low prevalence of gestational diabetes and impact of the new IADPSG recommendation

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Background and aims: IADPSG has suggested new diagnostic criteria for gestational diabetes (GDM). The impact of a universal screening strategy using an oral glucose tolerance test (OGTT) with more stringent diagnostic criteria for GDM has not been well defined in a population with a low background prevalence of GDM. The aim of our study was to evaluate the efficacy of our two-step screening strategy for GDM using a 50g glucose challenge test (GCT) and diagnosis for GDM based on the Carpenter and Coustan criteria using a 3-hour 100g OGTT and to evaluate the impact on GDM prevalence using the new recommendations.

Materials and methods: We retrospectively analyzed the two-step screening strategy for GDM from 2005–2010. The current policy in our university hospital advocates an universal screening with GCT between 24 and 28 weeks of pregnancy. We analyzed how many pregnancies received a GCT in our hospital and how many abnormal GCTs (≥ 140 mg/dl) were appropriately followed by an OGTT. We compared the GDM prevalence based on the two-step screening strategy with the GDM prevalence based on the new criteria.

Results: Over a six year period there were 12699 pregnancies (median number per year of 2090). Mean age of women was 30.8 ± 0.4 years, 90.3% were of Caucasian and 3.8% of Mediterranean descent. 17.1% were multiparous and 39% primigravid. 21.6% of women were overweight and 8.6% were obese. Of all pregnancies 53.0% (6726) received screening for GDM: 96.3% received a GCT and 3.7% an OGTT without previous GCT. Of the 6476 patients receiving a GCT, 16.4% (1065) had an abnormal test. Only 77.1% (821) of abnormal GCTs were followed appropriately by an OGTT. Of all OGTTs, 21% (222) showed GDM based on the Carpenter and Coustan criteria, leading to an overall prevalence of GDM of 1.7%. The prevalence of GDM in all 6726 tested pregnancies was 3.3%. The positive predictive value of GCT to have GDM was 20.3%. The number of pregnancies receiving an OGTT increased from 6.3% in 2005 to 12.3% in 2010 ($p < 0.0001$). The number of abnormal OGTTs, based on Carpenter and Coustan criteria, increased from 21.1% in 2005 to 25.8% in 2010 ($p = 0.312$). Using the new IADPSG criteria, 36.1% (382) of OGTTs showed GDM, leading to an overall prevalence of GDM of 3.0%. The prevalence of GDM in all 6726 tested pregnancies increased to 5.7%. Compared to diagnosis with the Carpenter and Coustan criteria, this led to a relative increase of 72.0% in the number of GDM diagnosed ($p < 0.0001$). The number of abnormal OGTTs based on the IADPSG criteria, increased from 36.2% in 2005 to 42.7% in 2010 ($p = 0.223$). Based on the IADPSG criteria, 36.1% of all GDM diagnosis was due to an elevated fasting plasma glucose.

Conclusion: Diagnosis of GDM in our hospital seems suboptimal as only half of the patients received a GCT and only two thirds of abnormal GCTs were appropriately followed by an OGTT. When the new criteria for GDM are used, the prevalence for GDM increases significantly but remains low despite the overestimation using the 100g OGTT. Advocating a universal screening strategy using an OGTT with the new criteria for GDM, might not be cost effective in a population with a low background prevalence of GDM. Risk based screening might be a more practical approach for such a population.

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Is there a seasonal variation in the incidence of gestational diabetes mellitus?

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Background and aims: Gestational diabetes mellitus (GDM) seasonability has been rarely addressed in literature, with conflicting results. The aim of the study is to evaluate monthly GDM incidence in a large group of Greek women examined during the past decade.

Materials and methods: 7618 pregnant women underwent a 3-hour, 100g OGTT during the 3rd trimester. For GDM diagnosis the ADA 2000 criteria were used. Seasonal and monthly GDM incidences as well as mean seasonal glucose levels during OGTT were calculated. Data for mean month temperature during the decade were obtained from the Hellenic National Meteorological Service. For the statistical analysis the following tests were used: χ^2 , odds ratio and MANOVA.

Results: GDM incidence, relative prevalence (RP) and odds ratio (OR-95%CI) per month, using January as a reference point, as well as mean monthly temperatures are shown in the Table.

Month	GDM%	RP	OR(95%CI)	Mean temperature C°
Jan	32.7%	1	relative to January	9.5
Febr	36.4%	1.11	1.21(0.94-1.56)	10.5
March	36.9%	1.13	1.24(0.97-1.58)	12
April	41.2%	1.26	1.42(1.11-1.82)	16
May	38.3%	1.17	1.31(1.03-1.65)	21
June	44.7%	1.36	1.60(1.26-2.04)	25
July	49.2%	1.50	1.88(1.49-2.38)	28
Aug	49.9%	1.52	1.84(1.42-2.37)	28
Sept	39.5%	1.21	1.41(1.11-1.79)	24
Oct	38.9%	1.19	1.29(1.02-1.65)	20
Nov	37.4%	1.14	1.25(0.98-1.59)	16
Dec	32.8%	1.00	1.02(0.79-1.32)	11.5

Seasonal GDM incidence was significantly different: Winter(W)= 28.1%, Summer(S)= 39.2%, Spring (Spr)= 32.4% and Autumn(A)= 32.4% ($\chi^2=51.0$, $p<0.0001$). The odds ratio for GDM incidence during summer was 1.65(95%CI:1.43-1.90), during spring and autumn 1.23 (95%CI:1.08-1.39) in relation to winter. Glucose levels (Glu) during OGTT were calculated. There was no statistical difference in fasting glucose blood levels. On the contrary, significantly increased blood glucose values were observed at 60', 120' and 180' in S vs W, while Spr and A values were intermediate (ANOVA: $p<0.0001$). Glu 60'(mg/dl): W= 162.8 \pm 41.9, Spr= 165.8 \pm 41.9, A= 166.9 \pm 39.4, S= 172.9 \pm 39.8, Glu 120'(mg/dl): W= 136.6 \pm 41.4, Spr= 137.9 \pm 40.6, A= 139.2 \pm 38.5, S= 146 \pm 40.3, Glu 180'(mg/dl): W= 108.6 \pm 33.2, Spr= 109.8 \pm 33.2, A= 111.1 \pm 32, S= 115.9 \pm 33.8. The effect of glucose on blood levels at 60', 120' and 180' remained an independent significant factor after adjustment for age, gestational age, BMI, weight gain during pregnancy, systolic and diastolic blood pressure (MANOVA model, $p<0.0001$).

Conclusions: GDM incidence in Greece presents seasonal variation. The risk for GDM diagnosis during summer is significantly increased (~70%) compared to winter. The differences in seasonal incidence are due to post glucose load levels variation, while no differences were found in fasting glucose levels. Whether the observed variations could be attributed to differences in ambient temperature (despite steady room temperature during the OGTT procedure), or other environmental and nutritional factors, remains to be clarified.

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Determinants of depressive symptoms in women screened for gestational diabetes 3 years after delivery

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Background and aims: Recently it was suggested that increased blood glucose per se is not associated with an increased risk of depression while screen detected diabetes may cause psychological distress and a higher rate of depression. However it is not clear whether screen detected gestational diabetes has any long term mental consequences. Thus we set out to assess the association between the severity of depressive symptoms and previous gestational diabetes (pGDM) or current glucose status and to describe other determinants of depressive symptoms 3 years after delivery.

Material and methods: In a case-control study nested within a cohort of 3001 pregnant women who took part in a universal GDM screening program and delivered between 2005-2006. All 193 pGDM patients and 191 randomly selected controls with normal glucose tolerance (NGT) were invited for a clinical examination. Altogether 77 pGDM and 40 controls participated. Data on anthropometrics, socio-economic status, and lifestyles were collected. Depressive symptoms were screened by the 21-item Beck Depression Inventory (BDI). Current glucose tolerance status was determined according to a 75 g oral glucose tolerance test.

Results: PGDM patients were older ([mean \pm SD] 35.5 \pm 4.1 vs. 33.5 \pm 3.7 yrs, $P=0.01$), had higher fasting and 2-hour blood glucose (5.7 \pm 1.2 vs. 5.2 \pm 0.4 mmol/l, $P=0.004$; 6.6 \pm 2.1 vs. 5.1 \pm 1.3 mmol/l, $P<0.0001$), had higher systolic blood pressure (120 \pm 18 vs. 113 \pm 10 mm Hg, $P=0.03$), and were more frequently diagnosed with glucose intolerance (28.6% vs. 0%, $P<0.0001$) than controls, while body mass index (BMI) (25.6 \pm 5.7 vs. 23.9 \pm 3.7 kg/m²; $P=0.086$) and BDI scores (median[IQR], 5.0[7.0] vs. 4.5[7.8], $P=0.51$) were similar. BDI scores were significantly related to current job status (employed $r=-0.22$, $P=0.03$), higher education ($r=-0.21$; $P=0.02$), height (-0.27 , $P=0.003$), BMI (0.25; $P=0.006$), net income (<50 - 8.5[10.8] / 50-100 - 6.0[8.0] / 100-150 - 4.5[6.3] / 150-200 - 2.5[5.8] / > 200 thousand HUF - 0.5[2.0], $P=0.005$), marital status (married - 3[7] / single - 7[7] / living with a partner - 10[10], $P=0.02$) but were independent of glucose tolerance status ($r=0.06$, P NS). In multiple linear regression with BDI-score as the outcome, depressive symptom severity was independently related to lower family income, living with a partner (compared to single or married) and higher BMI (all $P<0.05$).

Conclusion: BDI scores were independent of screen detected GDM status and current glucose tolerance ~3 years after delivery. The main determinants of depressive symptoms were present socio-economic and marital status and measures of obesity.

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Gestational diabetes mellitus in the Mediterranean region

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Background and aims: There is little available data on the prevalence and phenotype of GDM in populations in the Mediterranean region. The aim of this study was to characterise the phenotype of Mediterranean women at 24-32 weeks of pregnancy at risk of developing Gestational Diabetes Mellitus. (GDM) or suffering from GDM as diagnosed by a 75 g Oral Glucose Tolerance Test (OGTT).

Materials and methods: Participating centres in the Mediterranean region recruited 75-200 women per centre in the 24th-32nd week of pregnancy. The study protocol was approved by the relevant Research Ethics Committee in each participating country and informed consent was obtained from all study subjects. These women were assessed for the presence of risk factors such as elevated body weight, BMI, age, history of recurrent miscarriage, unexplained past stillbirth and macrosomia, irregular menses, need for assisted reproductive technology, hypertension (pre-existing, gestational or pre-eclampsia) and a family history of diabetes mellitus. The subjects subsequently underwent

a physical examination including height and blood pressure estimation, a 75 gram OGTT with a baseline fasting insulin level and HbA1c estimation. Insulin resistance was assessed using Homeostatic Model Assessment (HOMA-IR). Patients diagnosed with GDM were managed according to locally set protocols. Results are presented as mean \pm standard deviation. Comparisons of continuous variables between groups were made using independent-samples t-test and regression coefficients calculated categorical variables were compared using the Chi-squared test.

Results: 1210 patients from thirteen different Mediterranean countries were recruited to the study. 115 patients (9.5%) were identified as developing GDM using the ADA criteria, 256 (21.2%) according to WHO criteria and 341 (28.1%) using IADPSG criteria. Significant risk factors in this population for developing GDM were maternal age >35 years ($p=0.005$), pre-pregnancy BMI > 25 ($p=0.006$), diastolic BP > 90 mmHg ($p<0.0001$), PH of macrosomia ($p=0.006$) and FH of DM in first degree relatives (mother: $p<0.0001$; father: $p=0.02$; siblings: $p<0.0001$). These risk factors all had a high specificity but low sensitivity. Only pre-pregnancy BMI showed a moderate specificity (63.3%) and sensitivity (50.4%). Only fasting blood glucose showed any significant correlation with oGTT-AUC estimation ($r = 0.8$). There were no significant correlations established between BMI and the biochemical parameters (FBG, oGTT-AUC, HbA1c and HOMA-IR). Similarly no significant correlations were noted between the various biochemical parameters (HbA1c to insulin, HOMA-IR, oGTT-AUC; and FBG to HbA1c and HOMA IR).

Conclusion: The significant discrepancy in the prevalence of GDM depending on the diagnostic criteria used highlights the need for a consensus to be reached as to which GDM diagnostic criteria should be adopted in order to ensure best practice and best obstetric and neonatal outcomes without unnecessarily increasing the cost-benefit ratio.

Supported by: MGSD

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Pregnancy outcomes in women with gestational diabetes treated with metformin or diet alone

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Background and aims: Increasing evidence suggests that metformin may offer advantages in the management of gestational diabetes (GDM). We compared pregnancy outcomes in GDM women treated with either metformin or diet alone

Method: Prospective study of women attending our Diabetes Antenatal clinic. All patients received standardised dietary and exercise advice from a specialist dietitian. Patients performed home glucose monitoring aiming to achieve target glucose values (fasting <5.6mmol/l, 1h post-prandial <8mmol/l, 2h post prandial <7mmol/l). If 3 or more values were outside target range, treatment with metformin 500mg bd was added and the dose titrated up to a maximum of 2500mg/day. Pregnancy outcomes were recorded and results compared for diet or metformin-treated patients.

Results: We studied 266 GDM women (mean \pm SD); age: 33 ± 5.4 yrs) treated with metformin and 130 (age 33 ± 5.2) managed with diet alone. Women on diet alone had significantly lower body mass index (BMI) kg/m² (27 ± 6 vs 30 ± 7 ; $p<0.01$), HbA1c % (5.4 ± 0.5 vs 5.6 ± 0.7 ; $p<0.01$) and oral glucose tolerance test values (fasting glucose 4.8 ± 0.6 vs 5.2 ± 0.9 ; $p<0.01$; 2h glucose 8.6 ± 1.8 vs 7.9 ± 1.6 ; $p<0.001$). More women in the metformin group were induced at term (27% vs 16%; $p=0.03$), and were delivered by elective Caesarean section (19.9% vs 13.1%; $p=0.04$). Macrosomia (birth weight >90th centile) was dramatically reduced in metformin treated patients (11.3% vs 21.5%; $p<0.01$) as was small for gestational age (BW<10th centile) 7.9% vs 16.2%; $p=0.01$). Neonatal hypoglycaemia was less frequent in metformin treated mothers (7.1% vs 15.2%; $p<0.01$). Mean birth weight, admissions to neonatal unit, congenital malformations and shoulder dystocia rates were similar.

Conclusions: Despite greater glucose intolerance in the metformin group at baseline, neonatal outcomes were better compared with those on dietary measures alone. These results add to accumulating evidence that metformin is beneficial in the management of GDM.

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Association of gestational diabetes mellitus with dietary intake of macro- and micro-nutrients

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Background and aims: Gestational Diabetes Mellitus (GDM) is the commonest metabolic disorder of pregnancy and it substantially increases the maternal as well as fetal mortality and morbidity. It is well known that dietary practices are linked to glucose intolerance; however, the role of diet in the pathogenesis of GDM has not yet been adequately studied. The present study was undertaken to investigate the association of GDM and its basic defects with the dietary intake of few macro- and micro-nutrients.

Materials and methods: Under a case-control design 89 GDM subjects along with 101 pregnant controls were investigated for their age, BMI, blood pressure, glycemic status (fasting and 2 hr blood glucose, HbA1c), lipid profile (TG, Chol, LDL and HDL) and insulinemic status (C-peptide). GDM was diagnosed following the WHO Guideline. Dietary intakes of both the groups were assessed by Food Frequency Questionnaire. Serum glucose was measured by glucose oxidase method, lipids by enzymatic method and HbA1c was measured by HPLC-based autoanalyzer. Serum C-peptide was measured by chemiluminiscent 'ELISA' and insulin secretion (HOMA%B) and sensitivity (HOMA%S) were calculated by homeostasis model assessment. Data were analyzed by appropriate univariate as well as multivariate analysis

Results: Serum TG and C-peptide were found to be significantly higher in GDM group compared to control. HOMA%B did not differ between the groups, but HOMA%S was substantially less in GDM [Median (Range), 46 (26-90)] as compared to control [124 (49-299); $p<0.001$]. Total energy intake was about 1.5 times higher in GDM [Kcal, 2550 (1135-3675) as compared to control [1600 (1005-3700); $p<0.001$]. The higher calorie intake was contributed by all the 3 major energy sources is carbohydrate [g/day, GDM control 335(139-462) vs 200 (130-389); $p<0.001$], fat [60 (23-98) vs 20 (11-40); $p<0.001$], and protein [90 (70-199) vs 87 (70-196); $p=0.005$]; however, the proportionate rise of fat intake (about 3 times) was much higher in GDM compared to the other two nutrients. The intake calcium, iron, Vit B1 and Vit C did not differ between the two groups, but Vit B2 intake was significantly higher in GDM [μ g/day, 1.30 (0.20-2.78)] as compared to control [1.19 (0.34-2.74); $p=0.041$]. On logistic regression analysis GDM was found to be associated with total carbohydrate intake ($p<0.001$) and it was strongly associated with total fat intake ($p<0.001$). HbA1c and HOMA%S were also found to be strongly associated with total fat intake ($p<0.001$) on logistic regression analysis. On multiple regressions HbA1c showed significant association with Vit B2 intake ($p=0.018$).

Conclusion: In conclusion, GDM and its basic defects seem to have strong association with energy, fat intake and carbohydrate and riboflavin intake may also have some association with these defects.

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Vitamin D levels and glucose homeostasis in pregnancy

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Background and aims: Recent reports have described the non-classical effects of vitamin D and its deficiency has been associated to an increase in the risk of several pathologies. So far, however, data regarding the role of vitamin D in the development of gestational diabetes mellitus (GDM) are inconsistent. Our study evaluates maternal serum 25-hydroxy-vitamin D (25-OH-D) at 24-28 weeks of gestation and its correlation with glucose homeostasis.

Materials and methods: Measurements from 228 pregnant women (mean age 33 [29-36]) were taken at the time of O'Sullivan test during the months of June - September 2010 at the San Carlos Clinical Hospital in Madrid, Spain. When the O'Sullivan test was positive, a 100 g glucose-tolerance test (OGTT) was performed. Women were diagnosed as diabetic or non-diabetic using the Coustan and Carpenter criteria. Serum 25-OH-D and other parameters of glucose metabolism were assessed. A demographic and lifestyle questionnaire was performed.

Results: Mean serum 25-OH-D was 19.15 ± 9.16 ng/ml. Insufficiency (<30 ng/ml) and deficiency (<10 ng/ml) was reported in 92.2% and 15% of the participants, respectively. No significant differences in 25-OH-D levels (19.28 ng/ml vs. 19.13 ng/ml, $p=0.939$) or in the rate of insufficiency (95.8% vs. 91.7%, $p=0.481$) were found in diabetic vs. non-diabetic pregnant women. However, a correlation was observed between 25-OH-D levels and glucose homeostasis (table 1). Serum 25-OH-D was 21.34 ng/ml in European women and 16.71 ng/ml in South-American women ($p<0.0001$). GDM prevalence was greater amongst the first ones (18.3% vs. 6.2%, $p=0.025$), who were also older and presented a greater increase in weight.

Conclusion: Hypovitaminosis D is frequent during pregnancy. Low concentrations of serum 25-OH-D were associated to higher fasting serum glucose levels (FSG), 1h-OGTT glucose level, HbA1c values and homeostasis model assessment index (HOMA-IR). The role of vitamin D in GDM deserves further investigation.

Table 1: Values show median (Q1–Q3).

25-OH-D ng/ml (n)	<10 (32)	10–19.9 (98)	20–29.9 (74)	>30 (23)
FSG mg/dl	89 (84–93)	87 (82–92)	84 (79–89)	82 (79–87)
1h-OGTT mg/dl	162 (130–185)	155 (130–185)	152 (127–172)	145 (133–164)
HbA1c %	5.3 (5–5.4)	5.1 (4.9–5.3)	5.0 (4.9–5.3)	4.9 (4.9–5.2)
HOMA-IR	1.6 (0.9–2.7)	1.4 (0.8–2.9)	1.3 (0.9–2.9)	1.0 (0.6–1.7)

FSG: fasting serum glucose; HOMA-IR: homeostasis model assessment index equation (used as the insulin resistance index).

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Maternal vitamin D metabolism in gestational diabetes mellitus: a systemic review

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Background and aims: Vitamin D metabolism has been considered an important impact factor of type 1 and type 2 diabetes mellitus, while it remained unclear whether vitamin D impact the incidence of gestational diabetes mellitus (GDM). We are aimed to investigate the vitamin D metabolic status in GDM.

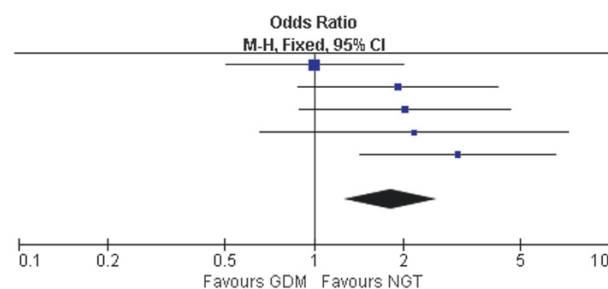
Materials and methods: We systemically collected observational studies of maternal vitamin D status in GDM from MEDLINE (1950–2010) and EM-Base (1950–2010).

Results: Six case-control studies have been finally included for a meta-analysis. Serum 25-(OH)-Vitamin D3 level, which describes the overall status of vitamin D, in pregnant women with GDM was significantly lower than those with normal glucose tolerance (NGT, overall MD -6.72 nmol/L, 95%CI -11.42 to -2.01 nmol/L, $P=0.005$). Vitamin D deficiency (serum 25-(OH)-Vitamin D3 < 50 nmol/L) was more frequently detected in GDM patients compared with normal pregnant women (overall OR 1.82, 95%CI 1.27 to 2.62, $P=0.001$). However, in pregnant women with impaired glucose tolerance (IGT), the decreased level of vitamin D (overall MD -8.86 , 95%CI -20.13 to 2.41 , $P=0.12$) and vitamin D deficiency (overall OR 2.40, 95%CI 0.79 to 7.29, $P=0.12$) did not reach statistical significance.

Conclusion: In conclusion, serum 25-(OH)-Vitamin D3 level and vitamin D deficiency is significantly associated with gestational diabetes mellitus, but not IGT in pregnancy.

Figure: Vitamin D deficiency is more commonly found in pregnant women with gestational diabetes mellitus (GDM) compared with those with normal glucose tolerance (NGT)

Study or Subgroup	GDM		NGT		Weight	Odds Ratio M-H, Fixed, 95% CI
	Events	Total	Events	Total		
Farrant, 2009	26	39	346	520	35.4%	1.01 [0.50, 2.01]
Clifton-Bligh, 2008	14	81	16	163	19.3%	1.92 [0.89, 4.16]
Soheilykhah, 2010	45	54	79	111	19.0%	2.03 [0.89, 4.62]
Maghbooli, 2008	49	52	465	527	10.6%	2.18 [0.66, 7.20]
Zhang, 2008	19	57	16	114	15.7%	3.06 [1.43, 6.57]
Total (95% CI)		283		1435	100.0%	1.82 [1.27, 2.62]
Total events	153		922			
Heterogeneity: $\chi^2 = 4.79$, $df = 4$ ($P = 0.31$); $I^2 = 17\%$						
Test for overall effect: $Z = 3.24$ ($P = 0.001$)						



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Vitamin D deficiency is related to the severity of gestational diabetes mellitus (GDM)

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Aim: In a previous study we found a significant relation between vitamin D deficiency and fasting glucose levels during pregnancy. The aim of the present study was to further evaluate the relation of vitamin D levels and GDM in a larger group, using the new IADPSG criteria.

Patients and methods: In a prospective study 348 Greek pregnant women aged 30 ± 5.7 years underwent a 75g OGTT in the third trimester of pregnancy (27.4 ± 4.2 w), during which serum 25(OH)D, PTH, Ca, and P concentrations were also measured. GDM was diagnosed using the IADSPG criteria (adopted by ADA in 2011). For 25(OH)D deficiency a cut-off point of 20 ng/ml was chosen. Age, height, pre-pregnancy weight, BMI and blood pressure (BP) were recorded. HOMA-IR was calculated. 25(OH)D was converted to its natural logarithm (Ln). For the statistical analysis we used: χ^2 , ANOVA, Pearson's linear correlation and multiple linear regression.

Results: 25(OH)D deficiency was found in 196 out of 348 pregnant women (56%). No difference was found between normal (20.1 ± 7.1 ng/ml) and GDM (19.7 ± 7.5 ng/ml) women ($p = 0.592$). However pregnant women with 3 abnormal glucose values in the OGTT ($n=35$) presented with significantly lower levels of 25(OH)D compared to those with one ($n=82$) or two abnormal values ($n=53$) and normal ($n=152$) pregnant women (16.3 ± 5.7 vs 20.9 ± 7.7 , 20.1 ± 7.9 , 20.1 ± 7.1 ng/ml respectively, $F=3.4$, $p<0.018$). The relationship remained significant after adjustment for BMI, age, gestational age and seasonal variation. In this group the odds ratio for 25(OH)D deficiency was 2.43 (95% CI: 1.1–5.4). Ln-25(OH)D was negatively correlated with fasting plasma glucose ($r = -0.108$, $p=0.047$). Pregnant women with fasting hyperglycemia presented with lower 25(OH)D levels ($p=0.027$). Additionally Ln-25(OH)D was negatively correlated with HOMA-IR ($r = -0.134$, $p<0.05$). Multiparous pregnant women (≥ 2 parities) presented with significantly higher prevalence of 25(OH)D deficiency compared to nulliparous and women with one parity (75% vs 56% and 51% respectively, χ^2 for trend: 7.938, $p = 0.019$). The odds ratio for 25(OH)D deficiency in multiparous women was 2.5 (95% CI: 1.2–5.2), adjusted for BMI. We confirmed the expected negative correlation of Ln-25(OH)D with PTH ($r = -0.285$, $p<0.01$) and also with systolic ($r = -0.114$, $p=0.037$) and diastolic BP ($r = -0.164$, $p=0.003$).

Conclusion: No association was found between 25(OH)D deficiency and the presence of GDM; however, we found a relation with the severity of GDM, as expressed by the presence of three abnormal glucose values. Interestingly, multiparous women (≥ 2 parities) presented with a threefold increased risk for being vitamin D deficient. These two findings have not been previously reported.

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Obesity induced by high fat diet before and during gestation: evaluation of maternal and offspring repercussions on metabolic parameters

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Background and aims: Obesity and metabolic syndrome are increasing worldwide in children. Long term effects on offspring metabolism of the fetal intrauterine environment due to “metabolic imprinting” are to be evaluated and physiopathologic mechanisms are still misunderstood. High Fat Diet (HFD) during gestation and lactation promotes obesity and insulin-resistance in rat offspring. We created a model of obesity and metabolic syndrome using a HFD containing 21.4% of fat, this to be closer to physiological clinical models. The aim of this work was to study the metabolic status of our model in females, in order to define the consequences of obesity, independently of hyperglycemia, on maternal and offspring pancreas.

Materials and methods: Fourteen female Wistar rats (200g) were randomized in two groups and fed, 3 months before mating, either with Normal Diet (ND) or with HFD. At the end of gestation the rats underwent either abdominal or normal delivery. After delivery, rats were fed with ND during the 3 weeks of lactation and weaning, until sacrifice at 6 weeks of life. Before mating, during gestation and lactation, metabolic parameters were assessed on mothers and offspring. We performed histological analysis of pancreatic islets in 6 weeks-old offspring.

Results: In mothers, weight gain was significantly higher in the HFD group after 2 months, associated with higher leptin concentrations (12.1 ± 4.9 vs 6.5 ± 2.5 ng/ml, $p = 0.04$). After 3 months, in HFD rats IpGTT showed significantly increased C-peptide levels (2155.9 ± 434.9 vs 1307.7 ± 295.8 pM, $p = 0.01$) and glycemia (1.2 ± 0.4 vs 0.7 ± 0.2 g/l, $p = 0.01$) whereas fasting glycemia staid comparable in the 2 groups (0.7 ± 0.1 vs 0.7 ± 0.1 g/l), reflecting a glucose metabolism abnormality and insulin resistance. After 16 days of gestation, IpGTT showed comparable glycemia at baseline and after stimulation and C-peptide levels tended to be higher in HFD (t0 637.4 ± 426 vs 390.2 ± 211 pM, and t60 2853.5 ± 1806 vs 2058.1 ± 1328 pM). At birth, offspring weight was lower in HFD, but took rapidly over to be comparable at 6 weeks (sacrifice). Glycemia was significantly lower at weaning in HFD offspring (0.9 ± 0.1 vs 1.1 ± 0.2 g/l, $p = 0.01$), but tended to increase at 6 weeks (1.4 ± 0.3 vs 1.3 ± 0.1 g/l, $p = 0.11$) associated with a tendency to lower C-peptide (562.1 ± 89.9 vs 713.8 ± 388 pM, $p = 0.45$). C-peptide/glycemia were respectively 382.8 ± 100 vs 495.3 ± 280 ,

$p = 0.32$. Preliminary results of histological pancreatic islets analyze showed a tendency to hyperplasia and hypotrophy in HFD offspring.

Conclusion: This model showed that, mild HFD induced obesity, a normal fasting glycemia and increased stimulated glycemia associated to increased C-peptide in female Wistar rats. As already published in other HFD models, glucose metabolism improved during gestation with comparable glycemia with controls, confirming our model as obesity and insulin resistant. HFD offspring glycemia and weight were lower at birth and increased to comparable levels at 6 weeks, associated with a tendency to lower C-peptide concentrations, corresponding to the histological islets anomalies found. Due to the poor carbohydrate composition of mother milk, offspring insulin secretion becomes significant only after weaning. The impact of mother's obesity on offspring pancreatic β -islets histology and oxidative stress remains to be evaluated.

Supported by: Vaincre le Diabète

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ATLANTIC-DIP: raised maternal body mass index (BMI) adversely affects maternal and foetal outcomes in glucose tolerant women classified using International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria

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Background and aims: Raised maternal body mass index (BMI), in association with hyperglycaemia is associated with adverse pregnancy outcome. Whether BMI has an independent effect on adverse pregnancy outcome is not clear. We aimed to investigate the effects of raised maternal BMI on pregnancy outcome in glucose tolerant women, classified using the IADPSG criteria.

Materials and methods: Prospective observational study of pregnancy outcome in a cohort of women attending an antenatal clinic recruited to a universal screening programme for gestational diabetes. Maternal outcomes included glucose, delivery mode, pregnancy induced hypertension (PIH), pre-eclampsia (PET), antepartum hemorrhage (APH) and postpartum hemorrhage (PPH). Fetal outcomes included birthweight, congenital malformation, fetal death, neonatal jaundice, hypoglycemia and respiratory distress. Analyses performed using stepwise logistic regression and decision trees. Analyses adjusted for maternal age, parity, cigarette smoking and ethnicity.

Results: Increasing maternal BMI was associated with adverse pregnancy outcomes: higher cesarean section rates, pre-eclamptic toxemia, pregnancy induced hypertension, increased birth weight and congenital malformation. The association of normal range glucose with adverse pregnancy outcome was weak and did not interact with the effects of raised BMI. A BMI threshold of 28 kg/m² was associated with a significant rise in adverse pregnancy outcome. Adverse obstetric outcome in association with raised BMI was greater in primiparous women.

Conclusion: Raised maternal BMI, within the overweight range, is associated with adverse pregnancy outcomes. These adverse effects of BMI occur independently of maternal glucose.

Maternal and Neonatal Outcomes: a $p < 0.01$; b $p < 0.05$

	Elective Caesarean Section	Emergency Caesarean Section	Pre Eclamptic Toxaemia	Pregnancy Induced Hypertension	Large for Gestational Age Birthweight	Macrosomia	Malformation
BMI (Scale Variable)	1-081 (1-052, 1-110)a	1-075 (1-044, 1-106)a	1-073 (1-034, 1-114)a	1-053 (1-017, 1-090)a	1-031 (1-007, 1-056)b	1-047 (1-025, 1-070)a	1-065 (1-003, 1-131)b
Normal	1	1	1	1	1	1	1
Overweight	1-611 (1-168, 2-223)a	1-611 (1-175, 2-209)a	1-728 (1-104, 2-706)a	2-117 (1-368, 3-276)a	1-372 (1-057, 1-780)b	1-446 (1-136, 1-842)a	1-824 (0-849, 3-916)
Obese	2-659 (1-852, 3-817)a	2-476 (1-713, 3-580)a	2-660 (1-607, 4-402)a	2-220 (1-342, 3-672)a	1-456 (1-067, 1-987)b	1-725 (1-295, 2-298)a	2-446 (1-038, 5-762)b
Grade I Obese	2-439 (1-629, 3-650)a	2-416 (1-615, 3-615)a	2-569 (1-486, 4-442)a	2-236 (1-289, 3-878)a	1-385 (0-975, 1-967)	1-731 (1-258, 2-383)a	2-024 (0-764, 5-364)
Grade II Obese	2-566 (1-439, 4-577)a	2-285 (1-196, 4-369)a	2-484 (1-053, 5-858)a	2-020 (0-889, 4-590)	2-031 (1-249, 3-302)a	1-943 (1-212, 3-114)a	3-542 (1-063, 11-797)b
Grade III Obese	4-893 (2-169, 11-035)a	3-920 (1-425, 10-782)a	3-793 (1-082, 13-301)a	2-203 (0-634, 7-657)	0-956 (0-408, 2-334)	1-396 (0-636, 3-063)	2-922 (0-356, 23-999)

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Predicting normal birth weight using serial foetal abdominal circumference measurements and maternal risk factors in gestational diabetes mellitus

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Background and aims: Our study aimed to determine the role for serial fetal abdominal circumference measurements (ACMs) in predicting normal birth weight and macrosomia (birth weight > 4kg) in pregnancies complicated by gestational diabetes mellitus (GDM).

Materials and methods: This prospective cohort study reviewed 137 women diagnosed with GDM on a 100g glucose tolerance test (GTT) who delivered a singleton pregnancy at term. All women had at least 2 ACMs taken in the third trimester. Those who delivered a macrosomic infant were compared to those who did not. The ACMs, maternal risk factors and fetal birth weight were recorded. We tested ACM trends and maternal risk factors both in combination and individually for associations with fetal birth weight using regression analysis. This data was used in predictive models.

Results: A total of 326 ACMs from 137 pregnancies were analysed. They differed significantly at each gestational age when comparing the macrosomic group (N=35) and non-macrosomic group (N=102). Women in the MG had significantly greater fasting glucose measurements on diagnostic GTT (6.6mmol/l \pm 0.7 Vs 5.9mmol/l \pm 1.0, $p=0.019$) and maternal weight at delivery (96kg \pm 17 Vs 90kg \pm 17, $p=0.039$), and were more likely to have a history of macrosomia (60% Vs 31%, $p=0.001$). Serial ACMs below the 50th centile could predict normal weight delivery with 100% positive predictive value. Serial ACMs below the 75th and 90th percentiles in non-obese women without a history of macrosomic delivery identified normal weight delivery with a 98% and 96% positive predictive value respectively. Serial ACMs above the 75th centile could predict macrosomia with a sensitivity of 74%.

Conclusion: Serial ACMs can predict normal weight delivery in GDM when used in a risk factor based model. Serial ACMs are more useful for predicting normal birth weight than macrosomia in GDM.

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Comparison of pregnancy outcome between obese and non-obese women with gestational diabetes intensively treated

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Background and aims: We sought to compare the maternal and foetal outcomes of gestational diabetes between obese women (body mass index (BMI) >30kg/m²) and non obese women.

Materials and methods: From 1999 to 2009, we recruited all women with gestational diabetes treated and followed-up in Pitié-Salpêtrière hospital. The management approach included: self-monitoring blood glucose 6 times/daily, diet therapy plus insulin if metabolic goals were not reached (fasting blood glucose levels < 90mg/dl and 2-hour postprandial blood glucose levels < 120mg/dl). Pregnancy outcome variables included weight gain during pregnancy, cesarean section delivery, macrosomic or large-for-gestational age infants and a composite outcome for all neonatal complications.

Results: One thousand and forty eight women were enrolled. We compared 288 obese women (BMI 33.5kg/m²) to 752 non obese women (BMI 24.2 kg/m²). Obese women were likely older (33.14 vs. 32.43, $p=0.05$), non Caucasian (61.4% vs. 60.2%, $p<10^{-4}$) and multiparous (75.6% vs. 64.4%, $p<0.001$). They had more previous macrosomic newborns (20.3% vs. 10.3%, $p<10^{-4}$), more previous cesarean section delivery (23.7% vs. 11.6%, $p<10^{-4}$) and more previous gravid hypertension (5.9% vs. 2.8%, $p=0.03$). They had higher fasting blood glucose (0.96g/l vs. 0.92g/l, $p<10^{-4}$). Obese women were likely treated earlier (28.7 SA vs. 31.1 SA, $p<10^{-4}$), and insulin-treated (33.3% vs. 22.8%, $p=0.001$). They gained less weight (9kg vs. 13kg, $p<10^{-4}$) and had more cesarean delivery section (44% vs. 29.1%, $p<10^{-4}$). Their newborns were more likely macrosomic (14.3% vs. 9%, $p=0.02$) or large-for-gestational age (20.9% vs. 13.3%, $p=0.005$) and had more neonatal complications (10.3% vs. 6.6%, $p=0.005$). Multivariate logistic analysis (comprising age, fasting blood glucose and previous obstetrical complications), for women without previous cesarean delivery, confirmed that obesity was an independent risk factor of cesar-

ean delivery (OR=2.0 [IC95% 1.2-3.5] $p=0.01$). For women without previous macrosomic newborns, obesity was an independent risk factor of large-for-gestational age (independent of fasting blood glucose) only for women who were not insulin-treated (OR=1.9 [1.1-3.5], $p=0.04$). For macrosomic infant, the risk was not significant ($p=0.21$).

Conclusion: Our study showed that obesity increased the maternal and foetal complications of gestational diabetes, although obese women had an earlier care and a closer follow-up. However, insulin may partly reduce the risk of large-for-gestational age newborns.

Characteristics and pregnancy outcome in obese and non obese women with gestational diabetes

	Obese (n=288)	Non obese (n=752)	p
Age (years)	33.14 (21-49)	32.43 (14-49)	0.05
BMI (kg/m ²)	33.5 (30-61)	24.2 (15.2-29.9)	<10 ⁻⁴
Insulin treatment (%)	86 (33.3)	155 (22.8)	0.01
Weight gain (kg)	9 (-13-35)	13(0-39)	<10 ⁻⁴
Cesarean section (%)	122 (44)	213 (29.1)	<10 ⁻⁴
Macrosomic infants (%)	40 (14.3)	66 (9)	0.02
Large-for-gestational age (%)	58 (20.9)	98 (13.5)	0.005
Neonatal complications (%)	28 (10.3)	47 (6.6)	0.05

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Children born to women with prior gestational diabetes are predestinated to be overweight at a young age

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Background and aim: Gestational diabetes mellitus (GDM) occurs as a complication in between 1 to 14% of all pregnancies and it is well known that the disease increases the risk for macrosomia and caesarean delivery. Additional to these early complications, it is recently described that children born to these mothers have a future risk of overweight and obesity. The aim of this study was to follow-up BMI in GDM children and in their siblings.

Material: Between 1995-2000 we had 204 pregnancies with GDM in our region in South Sweden and 114 of these accepted to participate in the study. Longitudinal data regarding weight and height of the offspring together with siblings born to women who had pregnancies complicated with GDM were collected from health care centres and schools. The body mass index (BMI) of these children was then compared to reference values from the background population in Sweden.

Results: When comparing BMI of children born after a GDM pregnancy with BMI in their siblings, there was no difference at any age. Index children and siblings were therefore merged together (n=153) and compared to Swedish reference BMI values (n=1318-3650). No difference was found at birth, at age 1-2 yrs the children to GDM mothers had significantly lower BMI in both sexes and thereafter without difference until the age of 4 yrs in girls and 6 yrs in boys when higher BMI were found in GDM children. At the age of 10 the mean BMI of girls (n=62) was 18.8 and in boys (n=70) 18.1 compared to 17.0 ($p<0.001$) and 16.9 ($p<0.001$) respectively in controls.

Conclusion: Children to mothers with GDM gained less in weight during the first years of life. At age 4-6 yrs weight increased and these children had significantly higher BMI compared to background population. It is most likely that this is due to life style habits of the families rather than prenatal factors, since the same BMI pattern was found in siblings. Lifestyle intervention should therefore have priority in these families.

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Influence of gestational diabetes mellitus on weight outcome in twin pregnancies

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Background and aims: Gestational diabetes mellitus (GDM) complicates about 8.8 % of all pregnancies and it is associated with increased risk of adverse perinatal outcomes, including increased birth weight. Multiple birth rate is about 3% of all births, and it has an increasing pattern in the last decades due to the rising maternal age and increasing use of fertility treatments. Twin preg-

nancies have a higher risk for most adverse outcomes compared to singletons. Although GDM is clearly associated with macrosomia, its influence on weight outcome (including macrosomia, growth restriction and growth discrepancy), in twin pregnancies is not clear. We aimed to evaluate the influence of gestational diabetes mellitus on weight outcome in twin pregnancies.

Materials and methods: 2976 women with GDM who attended the Diabetes and Pregnancy Unit of our hospital from 1986 to 2009 were included (2922 singleton and 54 twin pregnancies). GDM was diagnosed based on National Diabetes Data Group criteria. Spanish birthweight charts for singleton and twin pregnancies were used to ascertain the rate of large for gestational age infants (LGA) and the rate of small for gestational age infants (SGA). Infant weight birth ratio (actual weight at birth/50th percentile weight for sex and gestational age) was also used to assess weight outcome. Growth discrepancy was defined as more than 20% of birthweight difference between twin newborns. Pregnancy outcomes for singleton GDM and twin GDM pregnancies were compared using the independent-samples Student's *t* test for quantitative variables and the chi-square or Fisher exact test for categorical variables. The Mann-Whitney-U test was used for comparisons between the twin GDM pregnancies.

Results: Women affected by GDM with twin pregnancies did not significantly differ from singleton pregnancies concerning body mass index (24.1 ± 4.0 vs 24.8 ± 4.7 ; $p = 0.331$), risk factors at diagnosis (1.9 ± 0.9 vs 1.90 ± 1.04 ; $p = 0.827$), fasting glucose at diagnosis (88.5 ± 14.8 vs 90.5 ± 14.6 ; $p = 0.328$), treatment with insulin (38.9 vs 49.1 %; $p = 0.088$), insulin dose (0.33 UI/kg ± 0.16 vs 0.28 ± 0.14 ; $p = 0.174$), and HbA_{1c} achieved at third trimester (5.1 ± 0.4 vs 5.1 ± 0.5 ; $p = 0.592$). Women with twin pregnancies were older (34.4 ± 5.0 vs 33.0 ± 4.4 ; $p = 0.018$), gained more weight during pregnancy (13.8 ± 5.2 vs 9.8 ± 4.2 ; $p = 0.000$), had less parity (1.7 ± 0.7 vs 2.1 ± 1.2 ; $p = 0.000$), and less gestational age at delivery (37.1 ± 1.8 vs 38.8 ± 1.2 ; $p = 0.000$). There was no difference in the incidence of LGA infants in twin GDM pregnancies compared to singleton GDM pregnancies (13.0 vs 7.6 %; $p = 0.189$) nor in the incidence of SGA (13.0 vs 14.9%; $p = 0.847$). Infant weight birth ratio was similar for twin GDM pregnancies and singleton GDM pregnancies (0.98 vs 0.98; $p = 0.729$). 13% of all twins had weight discrepancy of >20%. Fasting glucose at diagnosis was similar in mothers with discordant and not discordant twins (88.7 ± 10.6 vs 88.5 ± 15.6 ; $p = 0.780$). HbA_{1c} achieved in third trimester was higher in mothers of discordant twins (5.3 ± 0.2 vs 5.0 ± 0.4 ; $p = 0.031$).

Conclusion: Gestational diabetes mellitus has the same impact on weight outcome in newborns from singleton and twin pregnancies. Moreover, poor glycaemic control during pregnancy may have an influence in growth discrepancy in twins. Therefore it is important to treat twin GDM pregnancies using the same standards we use for singleton pregnancies in order to avoid adverse outcomes.

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Low BMI at age 20 years predicts gestational diabetes independent of BMI in early pregnancy: Tanaka Women's Clinic Study

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Background and aims: Maternal obesity and weight gain since early adulthood are known predictors of gestational diabetes (GDM) in Western countries. However, their impact has not been evaluated well in Asia where mean BMI levels are generally lower than in Western countries. Rather, thinness due to the desire to be slim like fashion models has been a major health problem among East Asian women of childbearing age. Especially, Japanese women have been reported to strongly desire to be thin, even though they have a lower BMI than other ethnic groups. In fact, more than 20% of women in their 20s are underweight in Japan. We therefore examined associations of BMI at age 20 years (BMI_{20y}) and BMI change since the age 20 years with risk of GDM in Japanese pregnant women.

Materials and methods: Prospectively observed were 624 consecutive pregnant women without recognized diabetes before the pregnancy who initially visited the obstetric clinic before 13 weeks gestation. Weight at age 20 years was self-reported. Baseline height and weight measurements were obtained at the initial obstetric visit. We calculated BMI in each at the time of age 20 years

and baseline. GDM was diagnosed by the International Association of Diabetes in Pregnancy Study Group's criteria. Logistic regression was performed, adjusting for relevant covariates.

Results: Twenty-eight women developed incident GDM. Mean age of participants was 33.4 ± 3.7 y and mean gestation at the first visit was 8.0 ± 2.0 wks. Mean baseline BMI and BMI_{20y} were 19.8 ± 2.1 and 19.0 ± 1.7 , respectively. Mean the change in BMI from age 20 to baseline age (BMI change) was 0.8 ± 1.7 . By multivariate logistic regression analysis that included maternal age, parity, baseline BMI and BMI_{20y}, we observed a statistically significant inverse association between BMI_{20y} and GDM incidence (odds ratio (OR), 0.68; 95%CI, 0.51-0.92). Compared with women in the highest 3 quartiles of BMI_{20y} (BMI_{20y}≥18), women in the lowest quartile of BMI_{20y} (BMI_{20y}<18) had a 4.84-fold (2.03-11.57) higher risk for GDM. When the BMI change replaced BMI_{20y} in the model, BMI change was associated with an increased risk of GDM (OR 1.47; 1.09-1.98). Women in the highest quartile of BMI change who gained 1.85 or more units had a 3.05-fold (1.18-7.93) greater risk than those in the lowest 3 quartiles. When we focused on the threshold of risk of GDM, women with the lowest quartile of BMI_{20y} had a 6.30-fold (2.26-17.59) greater risk compared with women in both the highest 3 quartiles of BMI_{20y} and the lowest 3 quartiles of BMI change.

Conclusion: Although a subsequent BMI gain from age 20 was an independent risk factor for GDM, lower BMI_{20y} was more strongly associated with an elevated GDM risk.

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Gestational diabetes mellitus results in a higher prevalence of small for gestational age babies

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Background and aims: Gestational Diabetes Mellitus (GDM) is associated with increased foetal and maternal morbidity and mortality. Previous studies have shown that babies of diabetic mothers are more likely to be large for gestational age (LGA). This retrospective study aimed to assess whether the converse may also be true, that there may also a higher rate of small for gestational age (SGA) amongst babies of mothers with GDM.

Materials and methods: This retrospective study offered universal screening for GDM to pregnant women in 5 hospitals between 2007-2009. During this time 5,500 women underwent testing for GDM using a 75g Oral Glucose Tolerance Test at 24-28 weeks gestation. GDM was defined by the International Association of the Diabetes and Pregnancy Study Groups guidelines (IADPSG).

Results: The prevalence of GDM was 12.4%. 4.5% of babies were small for gestational age (SGA) at birth in live births. Babies of mothers with GDM were more likely to have SGA than babies of non-diabetic women, OR 1.5, $p = 0.03$, 95% CI {1.02-2.24}. Mean Body Mass Index (BMI) was lower in mothers of SGA babies than mothers of babies who were average (AGA) or large for gestational age (LGA), 26.3 compared to 27.1, $p < 0.0001$. Smoking (OR 3.1, $p = 0.000$) pre-eclampsia (OR 3.99, $p = 0.000$), gestational hypertension, low parity (OR 0.8, $p = 0.005$), non-Caucasian ethnicity were also predictive of SGA. These SGA babies had a worse clinical outcome, including; higher caesarean section rate, higher requirement for neonatal intensive care, higher rates of hypoglycaemia and respiratory distress. 76% of diabetic women were treated with insulin. Insulin treatment did not affect rates of SGA when compared with dietary management.

Conclusion: This study shows another important negative outcome associated with GDM. Further research is required to identify the causative factor(s).

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Clinical outcomes of patients with gestational diabetes mellitus who do not have typical risk factors

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Background and aims: The UK's NICE guidelines recommend screening for Gestational Diabetes Mellitus (GDM) only in women with risk factors: BMI >30 kg/m², previous macrosomic baby or GDM, family history of diabetes, and South Asian, black Caribbean and Middle Eastern ethnicity. This study

aimed to identify the proportion of patients with GDM without such risk factors, who would not be screened for GDM by NICE guidance, in our ethnically diverse urban population (23% African/Caribbean; 4% South Asian; 73 white European and “other”). We also evaluated the impact of “typical” risk factors on maternal and fetal outcomes; and of the impact of The International Association of Diabetes and Pregnancy Study Groups (IADPSG) guidelines on risk factor based screening for GDM.

Materials and methods: From a retrospective cohort analysis of 70 GDM women, delivered between January and December 2010, we ascertained the incidence of recorded risk factors; maternal outcomes (treatment requirement, maternal complications, mode of delivery and postpartum oral glucose tolerance test (OGTT) values) and fetal outcomes (macrosmia, small for gestational age (SGA), fetal complications and admission to neonatal intensive care unit (NICU)) to examine the impact of risk factors on outcomes. The effect of using the IADPSG 2-hour diagnostic value in the OGTT (8.5 mmol/l vs. 7.8 mmol/l from WHO criteria currently in use) on the proportion of women with no risk factors was also calculated.

Results: 15.7% of GDM women had no GDM risk factors and 27% of these required insulin therapy to achieve our glucose targets for pregnancy. Ethnicity (61.4%), family history of diabetes (50%), and BMI (47.1%) were the commonest risk factors associated with GDM in our population but ethnicity was the only risk factor in 15.4%. 37.1% of GDM women had BMI <25, and of these, 34.6% had no other risk factors. The incidence of fetal complications was not different between women without and with risk factors: hypoglycaemia (57.6 vs 54.5% $p=0.85$); neonatal jaundice (30.5 vs 36.4% $p=0.70$); respiratory distress (13.2 vs 18.2% $p=0.81$) and shoulder dystocia (1.7 vs. 0% $p=0.66$). The incidence of maternal complications was not statistically different between those with and without risk factors, (hypertension (0 vs. 10.2% $p=0.31$); hypoglycaemia (0 vs. 1.7% $p=0.66$) and polyhydramnios (0 vs. 1.7% $p=0.66$)). The number of maternal risk factors for GDM was significantly correlated with higher A1c at diagnosis ($r=0.319$; $p=0.007$) and at delivery ($r=0.289$; $p=0.015$). 27.3% of women with no risk factors, and 24.5% of women with 1 or more risk factors would not be diagnosed GDM using the 2-hr threshold recommended by IADPSG.

Conclusion: 15.7% of women with GDM have no predisposing risk factors. Children born to these women have a similar risk of complications as those with established risk factors and their diagnosis may have been missed or delayed if a risk factor based screening strategy were employed. A similar proportion of those with and without risk factors would be missed if the new IADPSG recommended 2-hr glucose threshold was used to diagnose GDM. These data suggest that in our population a risk factor based screening programme would put a significant proportion of babies at risk.

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The risk of postpartum maternal hyperglycaemia in women with gestational diabetes is reduced by breastfeeding

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Background and aims: Gestational diabetes (GDM) is associated with adverse fetal and maternal outcomes. It identifies women at risk of pre-diabetes, type 2 diabetes (T2DM) and cardiovascular risk in later life. Recent studies have suggested that breastfeeding may confer a beneficial effect on postpartum maternal glucose tolerance in both women with GDM and normal glucose tolerance (NGT) in pregnancy.

Materials and methods: We compared results from 300 women with GDM and 220 women with NGT according to IADPSG criteria using a 75g oral glucose tolerance test (OGTT) at 24–28 weeks gestation by repeating the 75g OGTT postpartum to reassess glucose status. We also tested for postpartum metabolic syndrome (MetS) according to international criteria. Binary logistic regression was used to identify maternal factors that increased the risk of persistent glucose intolerance. Postpartum lactation status was categorised as breastfeeding alone, bottle-feeding alone, or both.

Results: 520 women were tested. OGTT results were classified as normal (FPG<5.6 mmol/l; 2h<7.8 mmol/l) or abnormal (IFG; 5.6–6.9, IGT; 2h 7.8–11.0, IFG+IGT; T2DM FPG $\geq 7 \pm 2$ h ≥ 11.1). Six of 220 (2.7%) women with NGT in pregnancy had postpartum dysglycaemia compared to 57 of 300 women (19%) with GDM in pregnancy ($P<0.001$). Non-Caucasian ethnicity (OR 3.40, 95% CI 1.45–8.02, $P=0.005$), family history of T2DM (OR 2.14, 95% CI 1.06–4.32, $P=0.034$) and insulin use in pregnancy (OR 2.62, 95% CI 1.17–5.87, $P=0.019$) were all predictive of persistent dysglycaemia. MetS was present postpartum in 31 of 300 women (10.3%) with GDM compared to 18

(8.2%) of 220 women with NGT ($P=0.4$). The prevalence of persistent dysglycaemia was lower in women who breast-fed versus bottle-fed their babies, or employed both techniques (7.1% v 18.4% and 11.2%, respectively, $p<0.001$).

Conclusion: In this Irish population the prevalence of persistent glucose intolerance in women with GDM in pregnancy is 19% compared to 2.7% in NGT women. Breast-feeding confers a beneficial effect on postpartum glucose tolerance. The precise mechanism behind this association is unclear and requires further study.

Table 1: Prevalence of persistent postpartum dysglycaemia according to breast-feeding status

	Prevalence of persistent dysglycaemia (%)
Breast-fed only n=212	15 (7.1%)
Bottle-fed only n=201	37 (18.4%)*
Breast+bottle n=107	12 (11.2%)

* $P<0.001$

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Metabolic fingerprinting of gestational diabetes mellitus

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Background and aims: Metabolomics is a rapidly evolving discipline of the comprehensive measurement of ideally all endogenous metabolites in a biological fluid. LC-QTOF-MS with an electrospray interface has advantages of sensitivity, mass accuracy, and good potential for biomarker identification, what makes this technique very important tool in metabolomics. Gestational Diabetes Mellitus (GDM) is a growing problem and increasingly common complication of pregnancy, however pathogenesis of GDM is not yet fully recognized. Application of LC-MS based metabolic fingerprinting to study plasma profiles of healthy pregnant women in comparison to those with GDM and 3 months postpartum with GDM in pregnancy seems to be a good approach to find metabolites responsible for the evolution and complications of this disease.

Materials and methods: Serum of 20 pregnant healthy women mean age 26 yrs, 20 pregnant women with diagnosed GDM after a 2-h 75-g OGTT, mean age 29 yrs and 20 women 3 months postpartum with GDM during pregnancy, mean age 29 yrs. Data were collected in positive ESI mode in separate runs on a QTOF (Agilent 6520) operated in full scan mode from 50 to 1,000 m/z. A matrix data has been aligned and filtered. Each group has been filtered separately applying 90 % Filter by Flags, it means that masses present in at least 90 % (18 out of 20) of the samples passed the filtering. To select metabolites characteristic to each group we used Venn diagram.

Results: There was found 21 metabolites characteristic only to GDM in comparison to other groups. In the group of women 3 months postpartum we observed still 31 metabolites characteristic for GDM. Those masses were putatively identified using several databases like HMDB, Metlin, KEGG, and LipidMaps.

Conclusion: Our research suggests that identified compounds indicate for alteration of fatty acids amides, lipids, and carnitines metabolism in gestational diabetes which may be important in the gestational diabetes mellitus pathogenesis. With metabolomic approach we observed, that 3 months postpartum there are still changes in metabolism connected with previous GDM.

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Elevated serum selenium concentrations in gestational diabetics compared to control pregnant women: cause or consequence?

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Background and aims: High serum selenium concentrations were positively associated with the prevalence of type 2 diabetes according to recently published data from the Third National Health and Nutrition Examination Survey. Moreover, an intervention study from the US, in which diabetes was a secondary outcome in the parent trial, concluded that oral selenium supplementation may increase risk for type 2 diabetes. As the role of selenium in gestational diabetes (GDM) has not yet been investigated, the aim of this study was to compare serum selenium concentrations, serum insulin and the activity of the selenium-dependent enzyme glutathione peroxidase (GPX) in different blood compartments of gestational diabetic (n=61) and control pregnant women (n=45) between the 24th and 28th week of pregnancy.

Materials and methods: All blood samples were obtained when study participants were screened for GDM according to WHO guidelines. All study participants filled in a detailed questionnaire and reported their intake of vitamin supplements. Serum selenium concentration was measured by hydride generation atomic absorption spectrometry. Plasma, red blood cell and whole blood hemolysate total GPX activity were determined by an end-point direct enzyme-assay in the presence of reduced glutathione and cumene-hydroperoxide as co-substrates. For insulin measurements, an automated immunoassay based on electro-chemiluminescence was used. Statistical analysis was performed using the Wilcoxon rank sum test and logistic regression.

Results: There was no difference in age between the two groups of pregnant women. BMI was slightly higher in gestational diabetic women than in controls (25.3±5.9 vs. 22.9±4.2 kg/m², p=0.009). The intake of vitamin supplements was similar in both groups of pregnant women. Serum selenium concentrations were significantly higher in gestational diabetic (50.8±12.8µg/l) compared to control pregnant women (40.8±7.8µg/l, p=0.0009) and selenium levels showed a significant positive association with the incidence of gestational diabetes even when adjusted for age and BMI (p=0.000227, OR: 1.09, 95% CI: 1.04–1.14). Red blood cell GPX activity was higher in gestational diabetic than in control pregnant women (7.16±2.14 vs. 5.92±0.67 U/g protein, p=0.02). Serum selenium concentrations correlated with plasma GPX activity in control pregnant women (p=0.005) but not in gestational diabetics. Serum insulin levels, HOMA-IR, plasma and whole blood total GPX activity did not differ between the two groups of pregnant women.

Conclusion: Gestational diabetics have significantly higher serum selenium concentrations than control pregnant women at the time of diagnosis, before starting dietary modifications. As some prenatal vitamins contain selenium, their selection requires consideration after risk factors for GDM have been assessed in the first trimester of pregnancy. Further studies may determine whether elevated selenium levels are a cause or consequence of GDM and whether selenium may play a specific role in gestational diabetes.

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Plasma phospholipid fatty acid composition and desaturases indices in women with gestational diabetes mellitus before and after delivery

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Background and aims: Plasma phospholipid fatty acid (PPFA) composition is affected not only by dietary fat intake, but also by endogenous fatty acid metabolism, which is regulated by enzymes such as elongases and desaturases. Desaturating enzymes, stearoyl-CoA desaturase (SCD, also known as Delta-9 desaturase), and Delta-5 desaturase (D5D) modulate fatty acid composition and are associated with insulin resistance. SCD is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. D5D is a key enzyme in polyunsaturated fatty acid, such as arachidonic (AA, 20:4n-6) and docosahexaenoic (DHA, 22:6n-3), metabolism. During pregnancy there is increased demand for the polyunsaturated fatty acids, AA and DHA. Furthermore, in type 1 and 2 diabetes, the activity of D5D is impaired. Therefore, we investigated the possibility that, in gestational diabetes mellitus (GDM), fatty acid composition in plasma phospholipids and desaturases indices may be altered before or after delivery. Moreover, we measured some parameters of inflammation, such as C-reactive protein (CRP), and interleukin-6 (IL-6), and investigated their relationship with desaturase indices in GDM and control women.

Materials and methods: Venous blood samples were obtained from 22 women with GDM and from 23 controls, during the third trimester of pregnancy and 6 months after delivery. We used a validate food questionnaire to determine the intakes during and after pregnancy. PPFA composition was analysed by gas chromatography.

Results: Dietary assessment of the GDM and control women did not provide evidence of a difference in intake between the two groups. During pregnancy also no difference in PPFA composition was observed between the two groups, while 6 months after delivery a significant increase in stearic acid (18:0) was found in the GDM women compared with the control subjects (12.83±0.96 vs 13.98±1.37; p=0.005). The D5D activity increased 6 months after delivery in controls (2.66±0.79 vs 3.15±0.70; p=0.040), while remained unchanged in GDM. Conversely, SCD index appears significantly decreased after pregnancy in GDM in comparison with the third trimester (p=0.001) and also with controls (p=0.024). In the last ones SCD index remained unchanged after delivery. In regards to the relationship between inflammatory markers and desaturases, only SCD index was found significantly correlated to PCR and IL-6, both during the third trimester and after pregnancy in GDM women.

Conclusion: In our study, the PPFA composition during the third trimester of pregnancy does not appear different between control and GDM women, while after delivery there are only minimal changes. In contrast to other reports, the data from the current study do not provide evidence of the impairment of D5D in GDM. Another important finding of the present study is that SCD activity seems regulated not only by dietary, hormonal and environmental factors, as well known, but also by GDM. In addition, our results suggest a relationship between fatty acid metabolism and inflammatory marker modulation in GDM.

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White blood cell count is an independent predictor of gestational diabetes mellitus

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Background and aims: Subclinical inflammation and insulin resistance are thought to be involved in the development of type 2 diabetes. Much less is known about first trimester role of inflammatory markers as predictors of subsequent GDM.

Material and methods: Using the data collected during a population-based screening program of gestational diabetes (WHO criteria 1999) in Budapest, Hungary we undertook a case-control study nested within a cohort of all screened women. Cases were all GDM women delivered between 01.01.2005 and 31.08.2008 (n=397) controls were metabolically healthy women delivered between 01.01.2006 and 31.03.2006 (n=317). After the exclusion of women with missing covariates (n=179) and women with previous GDM (n=56) the final sample included 234 cases and 249 controls. We investigated the association between prepregnancy and early pregnancy markers and the risk of gestational diabetes.

Results: GDM women were older (31.7 ± 4.3 [mean \pm SD] vs. 30.0 ± 4.3 yrs, $p < 0.0001$), had higher body weight (66.0 ± 14 vs. 64.0 ± 11.2 kg, $p = 0.031$), BMI (24.2 ± 4.8 vs. 23.1 ± 4.2 , $p = 0.01$), fasting blood glucose (4.5 ± 0.6 vs. 4.3 ± 0.6 mmol/l, $p < 0.0001$), and WBC count (9.2 ± 2.0 vs. 8.5 ± 1.9 G/l, $p < 0.0001$) and had a lower stature (165.2 ± 6.0 vs. 166.5 ± 5.8 cm, $p = 0.016$). They presented more frequently with a positive family history of diabetes (29.1% vs. 7.7%, $p < 0.0001$). According to a multiple logistic model the independent predictors of GDM were WBC count (OR=1.20 / 1 G/l, 95%CI [confidence interval]: 1, 07-1.34), height (OR=0.965 / 1 cm, 95%CI: 0.93-1), fasting blood glucose (OR=1.55 / 1 mmol/l, 95% CI: 1.07-2.25), age (OR=1.08 / 1 year, 95% CI: 1.03-1.14), and a positive family history of diabetes (OR=4.73, 95%CI: 2.56-8.77).

Conclusion: We found that first trimester white blood cell count was an independent predictor of the development of gestational diabetes suggesting that subclinical inflammation may be involved in the development of gestational diabetes. Further epidemiological studies are needed to investigate whether markers of subclinical inflammation - together with other known risk factors - could improve early detection of gestational diabetes.

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1197

Suppressor of cytokine signalling 1 and 3 expression in fat and placental tissue from women with gestational diabetes

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Background and aims: The suppressor of cytokine signaling (SOCS) proteins are feedback inhibitors of signaling pathways induced by cytokines, hormones and growth factors. Moreover, experimental data suggest that SOCS expression is a determinant of basal insulin signaling and a potential mediator of cytokine-induced fat tissue insulin resistance. Elevated SOCS3 mRNA expression has been shown in subcutaneous adipose tissue obtained from obese subjects and patients with type 2 diabetes, whereas no data concerning SOCS expression in pregnant women with the disturbances of glucose tolerance are so far available.

Materials and methods: In the present study we measured SOCS1 and SOCS3, as well as interleukin-6 (IL-6), IL-8 and leptin mRNA expression in paired samples of subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and placental tissue obtained from 18 pregnant women with normal glucose tolerance (NGT) and 20 subjects with gestational diabetes mellitus (GDM), using RT-PCR.

Results: SOCS1 and SOCS3 mRNA were detectable in all samples studied but their expression in fat and placental tissue did not differ significantly between the women with and without GDM. The patients with GDM had significantly higher IL-8 mRNA expression in VAT than had the women with NGT ($p = 0.007$), whereas the expression of IL-6 and leptin mRNA did not differ markedly between the two groups. SOCS1 mRNA expression in placental tissue was significantly higher than in SAT ($p = 0.002$) and VAT ($p = 0.01$), while SOCS3 mRNA expression in placental tissue was significantly lower than in fat tissue ($p = 0.0002$). Stepwise regression analysis revealed that SOCS3 mRNA expression in SAT and VAT was significantly related to IL-6 mRNA expression ($\beta = 0.86$, $p = 0.01$, $R^2 = 0.21$ and $\beta = 0.89$, $p = 0.02$, $R^2 = 0.85$, respectively), whereas SOCS3 mRNA expression in placental tissue was significantly predicted by HLD-cholesterol level ($\beta = 0.48$, $p = 0.01$) and IL-8 mRNA expression ($\beta = 0.42$, $p = 0.045$, $R^2 = 0.58$).

Conclusion: In conclusion, our results do not show significant differences in SOCS1 and SOCS3 mRNA expression in adipose and placental tissue obtained from pregnant women with and without GDM. This might be explained by the lack of differences in the expression of IL-6, which seems to play an essential role in the induction of the genes encoding SOCS proteins.

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1198

Unfavourable cytokine profile and increase of oxidative stress during and after pregnancy in women with gestational diabetes mellitus

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Background and aims: Although gestational diabetes mellitus (GDM) may represent a transient form of a latent metabolic syndrome (MetS) that might become evident later, little evidence demonstrating an association between early cardiovascular disease (CVD) markers and GDM has been shown. The aim of the study was to identify the presence of early markers of metabolic and CVD, as proinflammatory cytokines and oxidative stress status, in patients with GDM during pregnancy and after delivery.

Materials and methods: We performed a prospective case-control study within a sample of a total of 126 pregnant women (63 with GDM, 63 controls), reassessing with intrasubject analysis 41 women with a history of GDM (cases) and 21 controls, in the postpartum period. We analyzed demographic data, perinatal and obstetrics results and the levels of cytokines [measured in plasma using a commercial kit for multiplex analysis (Luminex® 100 LINCO plex Kit, St Louis, MO, EEUU)] and the markers of oxidative stress and antioxidants status [measured in serum or plasma using a commercial kit (Cayman Chemical, Ann Arbor, MI, USA)], during 24th and 29th week of gestation and twelve months after delivery.

Results: In the univariate analysis, were found statistically significant differences in the pregestational body mass index (BMI) ($p = 0.001$), levels higher of TNF- α ($p = 0.002$), leptin ($p = 0.001$), lipoperoxides [LPO] ($p = 0.04$), and lower of adiponectin ($p = 0.04$), catalase ($p = 0.04$) and superoxide dismutase [SOD] ($p = 0.001$) between cases and controls. The cesarean rate was 40% in cases and 10% in controls ($p = 0.001$). Multivariate analysis that was performed using non-conditional logistic regression showed adiponectin and catalase having a protective effect against (OR=0.9, $p = 0.02$, OR=0.39, $p = 0.006$, respectively) and the BMI and LPO carried a significant risk for GDM (OR=8.4, $p = 0.01$, OR=2.44, $p = 0.034$, respectively). At 12 months postpartum, there were significant differences in BMI ($p = 0.013$), fasting glucose ($p < 0.001$), glucose 120 min ($p = 0.007$), HOMA ($p = 0.002$), leptin ($p = 0.023$) and catalase ($p = 0.01$) between cases and controls. In the intrasubject analysis, fasting glucose and glucose 120 min levels decreased significantly in cases ($p = 0.04$) and controls ($p < 0.001$), TNF- α levels significantly increased both in cases ($p = 0.001$) and controls ($p = 0.001$) as well as the LPO and catalase levels, which increased in cases ($p = 0.001$ and $p = 0.001$, respectively) and controls ($p = 0.001$ and $p = 0.001$ respectively).

Conclusion: Women with GDM have more risk to have increased CVD markers, expressed at higher levels of TNF- α , LPO and lower of catalase and SOD. This unfavorable cytokine profile and increased oxidative stress, which remains in the postpartum period, evidence that women with a history of GDM have a permanent state of metabolic dysfunction that would make them more likely to develop DM2 and CVD in the future.

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Lipid peroxidation is raised in gestational diabetes: correlation with glucose levels

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Background and aims: Overproduction of reactive oxygen species (ROS) and free radicals, reflected in an increased concentration of lipid peroxidation (LPO) products results in enhanced oxidative stress and can lead to several diseases. There are, however, few data on concentrations on alteration of LPO products in gestational diabetes mellitus (GDM).

Materials and methods: Fasting concentrations of malondialdehyde + 4-hydroxyalkenals (MDA + 4-HDA), as an index of LPO were measured in 50 women at 28 weeks of gestation. The study group was divided according to the results of 50g glucose challenge test (GCT) and 75g oral glucose tolerance test (OGTT): Controls (n=20): normal responses to both GCT and OGTT, Intermediate Group (IG) (n=15): false positive GCT, but normal OGTT and GDM group (n=15): abnormal both GCT and OGTT. Insulin resistance was assessed by HOMA and Insulin Resistance Index (IRI) derived from glucose and insulin levels during OGTT.

Results: There were no significant differences between the subgroups, regarding age ($p=0.67$) and BMI, both before ($p=0.54$) and during pregnancy ($p=0.47$). The principal differences between women with GDM and controls pertained to glucose levels at 120 minutes of OGTT, that fell within the range typical for impaired glucose tolerance in all but one subjects with GDM, while fasting glucose levels (albeit still higher than in controls, $p \leq 0.01$) were still within the reference range for all but one women with GDM. There was no difference in the estimates of insulin resistance assessed by IRI between the Controls and Intermediate groups (0.68 ± 0.25 versus 0.93 ± 0.29 , $p=0.23$). There was, however, a marked difference in value of IRI between the GDM and Intermediate group (1.67 ± 0.39 versus 0.93 ± 0.29 , $p=0.015$), and between GDM group and Controls ($p<0.001$). LPO concentrations [MDA+4-HDA(nmol/mg protein)] were significantly higher in women with GDM (64.1 ± 24.3 (mean \pm SD), 39.3 ± 23.1 , 47.0 ± 18.1 , for GDM, intermediate and normal glucose tolerance controls, respectively, $p<0.05$), but there were no differences in LPO concentrations between Controls versus Intermediates. There was a significant correlation between concentrations of LPO products and glucose levels at 120 minutes of OGTT ($r_s = 0.42$, $p = 0.009$).

Conclusion: Concentration of lipid peroxidation products are raised in women with gestational diabetes and correlate with glucose concentrations even within the range considered to represent impaired glucose tolerance in non-pregnant subject. Physiological significance of this phenomenon still remains to be elucidated.

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Circulating vascular endothelial growth factor receptor-1 (SVEGFR-1) and receptor-2 (SVEGFR-2) in euglycaemic gestational diabetes

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Background and aims: Gestational diabetes mellitus (GDM) is a pathological state of carbohydrate intolerance beginning or first recognized during pregnancy, when left untreated increases the risk of adverse pregnancy outcome. GDM is associated with a significantly increased incidence of preeclampsia (PE), a severe complication of pregnancy, which is also related to an imbalance between angiogenic and anti-angiogenic factors as the soluble Vascular Endothelial Growth Factor Receptor-1 (sVEGFR-1). There is a paucity on information on the role of the soluble Vascular Endothelial Growth Factor Receptor-2 (sVEGFR-2) but lower levels are linked inversely

proportional to the progression of pre-eclampsia. Thus the aim of our study was to investigate if the presence of GDM is related with changes in the serum concentrations of these factors.

Materials and methods: sVEGFR-1 & sVEGFR-2 levels were measured in serum of 12 women with GDM ($32,08 \pm 1,59$ yo) and 9 matched for BMI (before pregnancy), race and age nondiabetic mothers (NGD) ($29,5 \pm 1,84$ yo) by a multiple bead array by the means of internally colour-codes microspheres with fluorescent dyes excited by laser beams. Statistical analysis performed with unpaired t-test.

Results: All pregnancies were uncomplicated and normal newborns delivered. The GDM group demonstrated significantly higher serum insulin levels than NGD ($12,06 \pm 1,7$ vs $7,28 \pm 0,94$ μ IU/ml) ($p=0,02$), while at the same euglycemic levels indicating the presence of insulin resistance state for GDM mothers. GHbA1c levels of GDM were within normal limits ($4,5 \pm 0,4\%$). There was no difference in the BMI of GDM vs NGD before pregnancy ($25,92 \pm 1,2$ vs $24,25 \pm 1,9$) as well as for the weight gained during pregnancy between the groups ($10,96 \pm 1,5$ vs $13,8 \pm 3,6$ kg). Circulating concentrations of both sVEGFR-1 ($2239,03 \pm 170,5$ vs $1746,24 \pm 36,8$ pg/ml) ($p<0,004$) and sVEGFR-2 ($6759,52 \pm 1071,5$ vs $1230,92 \pm 234,2$ pg/ml) ($p<0,0004$) were significantly increased in the GDM group than NGD.

Conclusion: Our results indicate that differences in the circulating levels of sVEGFR-1 and sVEGFR-2 are already present in the uncomplicated gestational diabetes in comparison to normal pregnancy and the monitoring of a further derangement in the profile of the pro-angiogenic and anti-angiogenic stability may be considered essential in the early detection of the progression to gestational abnormalities.

1201

Women with previous gestational diabetes show impaired parameters one year before the onset of type 2 diabetes

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Background and aims: After delivery, women with previous gestational diabetes mellitus (pGDM), despite a frequent normalization of their glucose levels, are at increased risk of developing type 2 diabetes (T2D). For this reason, it is of paramount importance to assess whether there are indications in pGDM about their possible development of overt diabetes. Aim of this investigation was studying non-diabetic pGDM for 6 years after partum and identifying those parameters which may predict the onset of T2D one year before its occurrence.

Materials and methods: A total of 88 non-diabetic pGDM were studied with a 75g 2h-oral glucose tolerance test (OGTT) with measurements of glucose, insulin and C-peptide, immediately after partum and annually during the 6-year study period. Insulin sensitivity and beta cell function were analyzed through mathematical modeling that yields fasting (QUICKI) and dynamic (OGIS) insulin sensitivity indices, and beta cell sensitivity to glucose stimulus (BGS). If a subject was found T2D at any year, she did not undergo any further examination. In this study, we analyzed the main anthropometric and metabolic parameters of non-diabetic pGDM who became T2D (progressors, PR) one year before the transition, compared to those parameters of the women who remained non-diabetic (non progressors, NP).

Results: Only 14 women became T2D. One year before onset of diabetes, PR showed impaired parameters when compared (Wilcoxon rank-sum test) with NP (Table). PR were slightly older and with higher BMI. Glucose was markedly elevated in PR; insulin and C-peptide were also elevated. Insulin sensitivity and beta cell function were clearly reduced in PR, but this impairment was more manifest with BGS, than with QUICKI and OGIS.

Conclusion: 16% of pGDM became T2D within 6 years after partum. All anthropometric and metabolic parameters were impaired in PR one year before the onset of T2D, mostly those related to insulin secretion. This strongly recommends a thorough follow up analysis of parameters (especially glucose levels and beta cell function index) in women with pGDM in order to early prevent the development of diabetes.

Table	NP	PR	p-value
Age(years)	35.0±0.3	39.6±1.2	0.001
BMI (kg m ⁻²)	26.0±0.3	32.0±2.2	0.003
Fasting plasma glucose (mmol l ⁻¹)	4.97±0.03	5.64±0.19	<0.001
Fasting insulin (pmol l ⁻¹)	64.2±2.8	95.3±14.7	0.007
Fasting C-peptide (pmol l ⁻¹)	629±19	895±122	0.008
2h-plasma glucose (mmol l ⁻¹)	6.21±0.1	8.31±0.44	<0.001
QUICKI	0.180±0.001	0.164±0.003	0.001
OGIS (ml min ⁻¹ m ⁻²)	425±4	367±15	0.001
BGS (pmol min ⁻¹ m ⁻² mM ⁻¹)	108±3	63±9	<0.001
Anthropometric and metabolic parameters (mean±SE).			

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PS 110 Pregnancy: biomarkers and outcomes

1202

To establish trimester-specific reference ranges for glycated haemoglobin (HbA_{1c}) in pregnancy

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Background and aims: Diabetes in Pregnancy imposes additional risks to both mother and infant. These poor outcomes are considered to be primarily related to glycaemic control which is monitored longitudinally through pregnancy by means of HbA_{1c}. The correlation between HbA_{1c} levels with clinical outcomes emphasises the need to measure HbA_{1c} accurately, precisely and for data interpretation comparison to appropriately defined reference intervals. From July 1st 2010, the HbA_{1c} assay in Irish laboratories became fully metrologically traceable to the IFCC standard, permitting HbA_{1c} to be reported in IFCC units (mmol/mol) and derived DCCT/NGSP units (%) using the IFCC-DCCT/NGSP master equation (DCCT = Diabetes Control and Complications Trial, NGSP = National Glycohemoglobin standardisation program). The aim of this project is to establish trimester-specific reference ranges in pregnancy for IFCC standardised HbA_{1c} in non-diabetic Caucasian women. This will allow us to define the goal for HbA_{1c} during pregnancy complicated by diabetes.

Materials and methods: Following informed consent blood was collected from 234 pregnant and 36 age -matched controls into EDTA and Fluoride oxalate tubes for HbA_{1c}, haemoglobin and glucose measurement. Pregnancy trimester was defined as follows: T1 (up to 12 weeks), T2 (13 to 27 weeks), T3 (>28 weeks to term). The Menarini HA8160 automated haemoglobin (Hb) analyser was used to assay HbA_{1c}.

Results: Non-parametric analysis of the data was performed. The 95% IFCC HbA_{1c} (DCCT) reference interval for Controls (n=59) 29-37mmol/mol (4.8-5.5%), Trimester 1 (n=27) 36mmol/mol (4.6-5.4%), Trimester 2 (n=107) 25-35mmol/mol (4.4-5.4%) and Trimester 3 (n=110) 28-39 mmol/mol (4.7-5.7%). A statistically significant difference between the median HbA_{1c} concentration of the control and Trimester 2 subjects, p <0.0001 was determined (Mann-Whitney test).

Conclusion: As HbA_{1c} changes throughout pregnancy, trimester-specific HbA_{1c} reference intervals are required to manage diabetes in pregnancy appropriately.

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1203

Glycaemic control in post-pregnancy follow-up in type 1 diabetes women

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Background and aims: Tight glycaemic control is essential during pregnancy complicated by type 1 diabetes mellitus (T1DM) in order to improve the prognosis for a mother and a child. Most diabetic women with T1DM are able to significantly lower their glucose levels during the pregnancy. However, very few data exists on their glycaemic control after the delivery. The purpose of this observational study was to assess glycaemic control in T1DM women after the pregnancy.

Materials and methods: We examined medical records of 345 consecutive singleton pregnancies in women with pregestational T1DM that received medical care in Department of Metabolic Diseases, Krakow, Poland between 1999 and 2010. We found 213 subjects that received an intensive diabetes management program during pregnancy and had at least one follow-up visit with HbA_{1c} measurement after the delivery. We analysed HbA_{1c} level in the 1st trimester (reflecting pre- and early pregnancy periods), the 3rd trimester, and the last post-pregnancy follow-up measurement (mean 8.6 months ±15.4 post delivery).

Results: Mean age of examined women was 27.7 years \pm 4.6, duration of T1DM 12.0 years \pm 7.9. The mean initial HbA1c level was 6.9 % \pm 1.3. We observed a significant improvement of HbA1c level that reached 5.7 % in the 3rd trimester (5.7 % \pm 0.7; $p < 0.000001$). At the post-pregnancy follow-up, we noticed a substantial rise of HbA1c (by 1.1%). This was significantly higher than in the last trimester ($p < 0.00001$) but not different from the initial value ($p = 0.1$). Subjects that after pregnancy were on continuous insulin infusion ($n = 82$) showed smaller rise than those on multiple daily injections ($n = 131$) (mean 6.4 % \pm 1.0 vs. 6.9 \pm 1.6; $p = 0.0038$, respectively). When we analyzed data from the follow-up earlier than 12 months (179 measurements) and after this cut-off point (34 measurements), we found no difference in the HbA1c values (6.65 vs 6.94; $p = 0.3$).

Conclusion: Concluding, in this largest so far clinical observation, T1DM women showed substantial post-pregnancy deterioration in glycemic control. This deterioration seems to depend on the treatment method.

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1204

Higher levels of Atrial Natriuretic Peptide (ANP) are present in early pregnancy in type 1 diabetic women developing preeclampsia

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Background and aims: Preeclampsia is characterized by abnormal vascular development, trophoblast invasion and placentation in early pregnancy. The vasoactive factors Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP) are synthesized in cardiac tissue and are markers of cardiac overload. Higher levels of ANP and BNP have been demonstrated in late pregnancy with preeclampsia. We investigated whether higher levels of ANP and BNP are present in early pregnancy in women with type 1 diabetes who subsequently develop preeclampsia.

Materials and methods: Observational study of 84 consecutive pregnant women with type 1 diabetes (median duration 15 years (range 1–32) and HbA_{1c} 6.7% (4.9–10.5) in early pregnancy). At 9, 14, 21, 27 and 33 weeks blood was sampled for measurements of ANP and BNP. HbA_{1c}, blood pressure and 24-hour urinary albumin excretion (UAE) were recorded. Based on UAE at inclusion, the women were classified as having normoalbuminuria (UAE <30 mg/24 hour), microalbuminuria (UAE 30–299 mg/24hour) or diabetic nephropathy (UAE \geq 300 mg/24 hour). Preeclampsia in women with normoalbuminuria or microalbuminuria was defined as blood pressure >140/90 mmHg and proteinuria \geq 300 mg/24 hour after 20 weeks. In women with diabetic nephropathy, the diagnosis was based on the same findings accompanied by a sudden increase of \geq 15% in systolic or diastolic blood pressure.

Results: Preeclampsia developed at median 34 (range 33–35) weeks in six women (7%) characterized by higher levels of ANP at 9 weeks (6.0 (1.6–7.8) pmol/l vs. 2.9 (0.7–7.8), normal range 3.6 \pm 1.4 pmol/l, $p = 0.02$), higher occurrence of diabetic retinopathy (100% vs. 55%, $p = 0.04$) and higher occurrence of diabetic nephropathy at 9 weeks (50% vs. 4%, $p = 0.004$) compared with women without development of preeclampsia. At 9 weeks, ANP levels were positively correlated with UAE (per 100 mg increase/24 hour, $r = 0.36$, $p = 0.0009$). BNP levels at 9 weeks were comparable between women with and without development of preeclampsia (5.1 (2.0–16.7) pmol/l vs. 3.6 (0.5–11.2), normal range 2.3 \pm 1.7 pmol/l, $p = 0.18$). Throughout pregnancy ANP levels were 34% higher ($p = 0.02$) in women with development of preeclampsia compared with women without development of preeclampsia whereas BNP levels were comparable between the two groups ($p = 0.26$).

Conclusion: In women with type 1 diabetes, higher ANP levels are present as early as at 9 weeks and in the remaining part of pregnancy in women with development of preeclampsia. This suggests that cardiac overload in pregnancy may play a role in the pathogenesis of preeclampsia.

1205

Incidence of thyroid dysfunction in pregnant women with type 1 diabetes mellitus living in iodine deficient area

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Background and aims: The interaction between pregnancy, diabetes mellitus and thyroid disturbance needs a particular attention. The antithyroid antibodies are more frequent in pregnant women with type 1 diabetes mellitus (DM) than in normal pregnant women. Beside, an increased prevalence of subclinical thyroid dysfunction has been described in pregnant diabetic women. The aim of this study is to verify if women with type 1 DM have more probability than normal women to develop a thyroid pathology in pregnancy, in an area with sufficient iodine.

Materials and methods: Ninety-six consecutive pregnant women with type 1 diabetes with age 20–38 years, and 25 healthy women, with the same age. Patients were evaluated at the following time-intervals: 9–12 and 18–20 weeks' gestation, at delivery and six months after delivery. We evaluated whether the presence of thyroid peroxidase autoantibodies (anti-TPO) was associated with changes in thyroid function, metabolic control and pregnancy outcome. At 8, 14, 21, 27 and 33 weeks, the diabetic women self-monitored plasma glucose (SMPG) (8/day) for 3 days and had blood samplings obtained.

Results: No significant difference was observed between diabetic and normal women, for the values of TSH ($p < 0.2$), FT4 ($p < 0.7$), FT3 ($p < 0.6$). Instead a significant difference was found between the thyroid volume ($p < 0.04$), in the diabetic patients versus the normal women, at delivery and six months after delivery. Anti-TPO was detected in 31 (32%) of the pregnant diabetic women compared with two women (8%) in the healthy controls ($p = 0.015$). The presence of anti-TPO was associated with higher TSH at 8 ($p < 0.0001$) and 14 weeks ($p < 0.05$) and lower free T4 at 8 weeks ($p < 0.05$) compared with anti-TPO negative women. Twenty untreated anti-TPO positive women had higher TSH compared with untreated, anti-TPO negative women ($p < 0.05$), but comparable free T4. Sixteen women (17%) were treated for thyroid disorder during pregnancy. No differences were detected between the diabetic women with and without anti-TPO regarding HbA_{1c}, insulin dose, median SMPG or pregnancy outcome. The results of this study underline the importance of the screening of the thyroid function and morphology, in all the pregnant women and, particularly, in the diabetic patients, to find the presence of glandular alterations as early as possible.

Conclusion: Anti-TPO was present in one-third of pregnant women with type 1 diabetes and associated with slightly higher TSH, but not poorer glycaemic control or adverse birth outcome. A total of 17% of women with type 1 diabetes mellitus were treated for thyroid disorder during pregnancy.

1206

Maternal glucose levels in early pregnancy and the risk of allergy in infancy: the mother child rhea cohort in Crete, Greece

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Background and aims: Prenatal maternal-fetal interactions and early-life programming might be associated with the development of asthma or allergic diseases. A number of studies suggest an association of childhood obesity with early manifestations of atopy, such as atopic eczema, and asthma. However the possible role of intrauterine exposure to hyperglycemia in the pathogenesis of allergic diseases in early childhood remains poorly understood. The purpose of this study was to examine the relation of maternal glucose and insulin levels in early pregnancy with the risk of atopic eczema, persistent wheezing, and diagnosis of allergy in early childhood.

Materials and methods: The mother-child "Rhea" study in Crete is a prospective cohort examining pregnant women (Greek and immigrants) residents at the prefecture of Heraklion that became pregnant during one year starting in February 2007 and initiated prenatal care before 15 weeks of gestation (mean: 12 weeks). Five hundred and seventy five pregnant women and their children were included in the analysis. Maternal fasting serum samples were collected at the time of the first major ultrasound (Mean: 12 weeks, SD: 1.5). Pregnant women were screened for gestational diabetes mellitus (GDM) between 24

and 28 weeks of gestation, and GDM was defined by the criteria proposed by Carpenter and Coustan. The primary outcome variables were physician-diagnosed atopic eczema, allergy, and persistent wheezing defined as two of more episodes of wheezing in the first year of life lasting more than three days. Multivariable log-binomial regression models were used after adjusting for the following confounders: offspring sex, birth weight, breastfeeding duration, maternal age, maternal education, parity, maternal pre-pregnancy BMI, parental asthma, and parental atopic eczema.

Results: Among the 575 children, 14.6% had been diagnosed with atopic dermatitis, 7.9% with allergy, and 7.9% with persistent wheezing in the first year of life. Overall, 8.1% of the mothers had GDM. An elevation of 10mg/dl in fasting glucose levels in early pregnancy increased the relative risk for atopic eczema by 28% (RR=1.28, 95 percent CI: 1.06, 1.55), while a 20mU/ml increase in fasting insulin levels increased the risk for atopic eczema by 14% (RR=1.14, 95 percent CI: 1.02, 1.28). Maternal glucose and insulin levels in early pregnancy showed no association with persistent wheezing in the first year of life. GDM was not found to be significantly associated with atopic dermatitis, allergy, or persistent wheezing in the first year of life.

Conclusion: These findings suggest that elevated glucose and insulin levels in early pregnancy increase the risk of atopic dermatitis in the first year of life. Further follow up of this cohort will allow determining if maternal hyperglycaemia has, an effect on asthma and allergy in later childhood.

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1207

The effect on clinician practices of the initiation of a new management programme for diabetes in pregnancy

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Background and aims: Following initial assessment of outcomes for women in our unit when NICE and ADIPS guidelines were instituted (reported at EASD 2010) we assessed what change if any had occurred in clinician management.

Materials and methods: We reviewed and compared the actions of clinicians prior to (pre 2008 cohort) and after the institution of the above guidelines (2008–2009 cohort). This consisted of routine fetal heart rate (FHR) monitoring at 36 weeks and growth ultrasounds at 28, 32 and 36 weeks gestation; insulin requiring women with unstable control were delivered at 39 weeks and those with optimal control were delivered at 40 weeks; and women with diet controlled gestational diabetes mellitus (GDM) were delivered by 41 weeks. The latter were followed throughout pregnancy in a routine antenatal clinic with review by obstetricians, diabetes educators, and dieticians. From the OBSTETRIX database data were obtained from 7 October 2006 until 31 December 2009.

Results: There were a total of 601 women; 211 women (pre new guidelines) and 390 in 2008–2009 (post new guidelines). The significant difference was that those women with insulin-controlled diabetes in pregnancy had a later gestation at delivery (P=0.036) showing that clinicians were undertaking a more conservative approach to induction of labour (IOL) in this group of women. There was a trend to a reduction in rates of IOL between the two groups 57% vs. 44% but this did not reach significance (P=0.059) and a trend to reduction in instrumental deliveries (P=0.055). As noted in our previous report the majority of women in the second cohort laboured spontaneously. There were no differences between the two groups in birth weights >4000g; or rates of shoulder dystocia, caesarean section, instrumental delivery, admissions to the nursery (NICU and SCN) and Apgar scores ≤7 between the two cohorts.

Conclusion: The institution of the newer guidelines resulted in later delivery and IOL in the women requiring insulin. There were no increases in adverse outcomes with the change of management.

1208

Maternal glycaemic control and hypoglycaemia in pregnancy: a randomised trial comparing insulin detemir with NPH insulin in 310 subjects with type 1 diabetes

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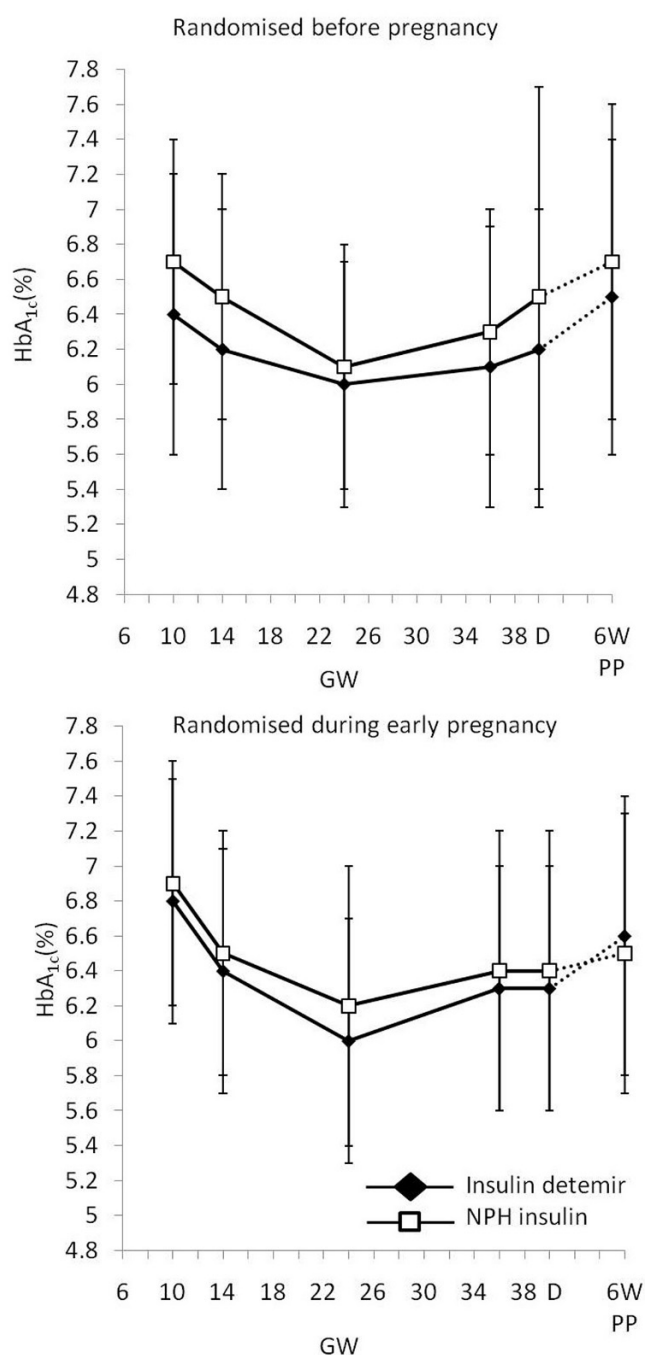
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Background and aims: Rigorous data investigating basal insulin analogues in diabetic pregnancy are lacking. The aim of this prospective, randomised, controlled, parallel-group, open-label trial was to compare the efficacy and safety of insulin detemir (IDet) vs. NPH (both with prandial insulin aspart) in pregnant women with type 1 diabetes (T1DM).

Materials and methods: T1DM women (HbA1c ≤8 % at pregnancy confirmation) were randomised to IDet (n=152) or NPH (n=158) up to 12 months before pregnancy or during pregnancy at 8–12 weeks gestation. The primary objective was to show that IDet was non-inferior to NPH for HbA1c at 36 gestational weeks (GWs) (primary endpoint). Non-inferiority was shown if the upper limit of the 95% CI for the treatment difference of IDet vs. NPH was below the pre-specified non-inferiority margin of 0.4% for both the Full Analysis Set (FAS) and Per Protocol Set (PP). The data were analysed using linear regression with effects of treatment, country and pregnancy status at randomisation, HbA1c at randomisation and the HbA1c at randomisation by pregnancy status at randomisation interaction.

Results: 79 and 83 women in the IDet and NPH groups, respectively, were pregnant at randomisation while 73 and 75 women, respectively, became pregnant following randomisation. Mean±SD demographics were: age 30.1±4.4 yrs; BMI 24.8±4.1 kg/m²; HbA1c 7.01±0.79%; fasting plasma glucose (FPG) 5.94±3.25 mmol/l and diabetes duration 12.3±8.0 yrs. For FAS, the estimated HbA1c at GW36 was 6.27% for IDet and 6.33% for NPH. IDet was shown to be non-inferior to NPH and not superior (FAS: -0.06, 95% CI: -0.21; 0.08; PP: -0.151; 95% CI: -0.34; 0.04). Patients reaching HbA1c ≤6.0% at both GW 24 + 36 was higher with IDet (36%) vs. NPH (29%), p=NS. Estimated FPG was significantly lower with IDet vs. NPH at GW 24 (5.38 vs. 6.32 mmol/l, difference -0.94 [-1.67; -0.21], p=0.012) and at GW 36 (4.76 vs. 5.41 mmol/l, difference -0.65 [-1.19; -0.12], p=0.017). There were no statistically significant or clinically relevant differences in hypoglycaemic events between treatments. The rate of major hypoglycaemia (events/yr) was 1.1 for IDet vs. 1.2 for NPH. There was no difference between groups in weight gain during pregnancy (11.5 kg and 11.0 kg, respectively). Mean doses of basal and bolus insulin increased during the 2nd and 3rd trimester and decreased after pregnancy, with no differences between treatments.

Conclusion: Lower FPG, but comparable HbA1c in late pregnancy was obtained using insulin detemir in comparison to NPH insulin in women with type 1 diabetes. Figure. Mean±SD HbA1c during pregnancy by timing of randomisation. D=delivery; 6WPP=6-weeks postpartum.



Clinical Trial Registration Number: NCT00474045

Supported by: Novo Nordisk

1209

Perinatal outcomes in pregnancy: a randomised trial comparing insulin detemir with NPH insulin in 310 subjects with type 1 diabetes

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Background and aims: The use of long-acting insulin analogues before and during pregnancy in type 1 diabetes is increasing, but their efficacy and safety remains uncertain. The aim of this prospective, randomised, controlled, par-

allel-group, open-label trial was to compare the efficacy and safety of insulin detemir (IDet) vs. NPH insulin (both with mealtime insulin aspart) in pregnant women with type 1 diabetes.

Materials and methods: Pregnant women with type 1 diabetes (age ≥ 18 yrs, HbA_{1c} $\leq 8\%$ at pregnancy confirmation) were randomised to IDet (n=152) or NPH (n=158) either before (up to 12 months) pregnancy (n=148) or during pregnancy (8–12 weeks gestation) (n=162). Pregnancy outcomes included a composite endpoint comprising: live born infants with birth weight $<10^{\text{th}}$ or $>90^{\text{th}}$ percentile for gestational age (GA) and sex; preterm delivery (<37 gestational weeks (GWs)); early fetal demise (<22 GWs); perinatal mortality; neonatal mortality; presence of major congenital malformations. Other endpoints included: live born infants; neonatal hypoglycaemia (PG <1.7 mmol/l within 24 hours of delivery); and adverse events (AEs) in offspring. The data were analysed using logistic analysis with treatment and pregnancy status at randomisation as covariates.

Results: There were 152 and 160 pregnancies in the IDet and NPH groups, respectively (2 women in the NPH group had a miscarriage and became pregnant again, without withdrawing). 25 pregnant women withdrew from the trial (10 in the IDet group vs. 15 in the NPH group); therefore pregnancy outcome is reported for 142 and 145 women, respectively. 89 (62.7%) of IDet vs. 96 (66.2%) of NPH-treated subjects experienced at least one endpoint in the composite outcome (odds ratio (OR) IDet/NPH: 0.86 [95% CI 0.53; 1.40], $p=0.551$). Maternal and neonatal outcomes for live born children were similar between the two groups (Table). 17 children (8 IDet/9 NPH) had congenital malformations. There were 2 perinatal deaths in the IDet group and 1 in the NPH group. 15 vs. 24 children in the IDet and NPH groups, respectively, experienced neonatal hypoglycaemia within 24 hours of delivery. There was no difference in the incidence of adverse events in the offspring between the two groups (37% IDet/35% NPH) or in the number of AEs/child (IDet 2.2/ NPH 2.7).

Conclusion: IDet is as well-tolerated as NPH with respect to perinatal morbidity and mortality when administered during pregnancy to subjects with type 1 diabetes.

Pregnancy assessments for live born children.		
	IDet	NPH
Pregnancy outcomes within the trial n	142	145
Live births, n	128	136
Birthweight (g) mean \pm SD	3504 (645)	3571 (601)
Birth length (cm), mean \pm SD	51.2 (3.9)	51.7 (3.4)
GA at delivery (weeks), mean \pm SD	38.2 (1.9)	37.8 (1.5)
Preterm delivery (<37 weeks), n (%)	26 (20.3%)	36 (26.5%)
Small for gestational age ($<10^{\text{th}}$ percentile), n (%)	3 (2%)	1 (1%)
Large for gestational age ($>90^{\text{th}}$ percentile), n (%)	59 (46%)	73 (54%)
Macrosomia (>4000 g), n (%)	24 (19%)	35 (26%)

Clinical Trial Registration Number: NCT00474045

Supported by: Novo Nordisk

1210

A meta-analysis of maternal outcomes in pregnant women using insulin glargine compared with NPH insulin

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Background and aims: Studies of insulin glargine in pregnancy are of small numbers of women. We aimed to provide quantitative estimates of maternal outcomes in women treated with insulin glargine versus NPH insulin during pregnancy.

Materials and methods: A literature search from multiple sources identified 49 articles related to insulin glargine and pregnancy. Eight articles that reported results of observational studies of diabetes pregnancies (preexisting or gestational diabetes) on at least 15 women each on insulin glargine and NPH insulin were included in the meta-analysis. Data from these studies were extracted and synthesized using methodology recommended by Cochrane Reviews with the software tool Review Manager 5.0. Mantel-Haenszel odds ratios were determined for all dichotomous outcome data using a random

effect model. Mean differences were determined for all continuous outcome data using an inverse variance method with a random effect model.

Results: The studies comprised a total of 702 women treated with either insulin glargine ($n = 331$) or NPH insulin ($n = 371$). Mean maternal age, body weight and body mass index were not different between the two groups of women using insulin glargine or NPH insulin. Women taking insulin glargine had diabetes 1.1 year (95% CI: 0.3, 2.0) longer than women taking NPH insulin. Maternal outcome measures including weight at delivery, weight gain, first and third trimester HbA_{1c} and incidence of hypoglycaemia, preeclampsia, cesarean section and gestational / new-onset hypertension were not different for insulin glargine versus NPH insulin (Table).

Conclusion: Published studies of insulin glargine versus NPH insulin in pregnancy give no signal of concern for insulin glargine regarding the maternal outcome measures evaluated. However, because quantitative estimates of difference for individual measures have large uncertainties, accumulation of larger data sets is now warranted. Close monitoring during pregnancy and just after birth is highly recommended for women with diabetes who are taking insulin.

Mean of differences across studies (insulin glargine - NPH insulin)				
Outcome	Studies/ Women (n/n)	Mean difference*	Odds ratio	95% CI
Weight at delivery (kg)	4/346	-0.82	—	-6.79, 5.15
Weight gain (kg)	5/495	0.16	—	-1.03, 1.35
HbA _{1c} - first trimester (%)	4/301	-0.08	—	-0.64, 0.49
HbA _{1c} - third trimester (%)	6/538	-0.01	—	-0.07, 0.05
Maternal hypoglycaemia - severe	5/472	—	0.84	0.18, 3.79
Pre-eclampsia	8/702	—	0.55	0.23, 1.32
Cesarean section	6/608	—	1.04	0.72, 1.52
Gestational / New-onset hypertension	4/360	—	0.49	0.20, 1.20

CI, confidence interval; HbA_{1c}, haemoglobin A_{1c}

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1211

Visual evoked potentials and psychomotor outcome in children of type 1 diabetic mothers, 3 years after delivery

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Background: The exposure to diabetic environment during pregnancy may have negative effects on brain functions of the offspring. Evoked potentials are sensitive indexes of central nervous system function.

Aim: to analyze maturation of Visual Evoked Potentials (VEPs) and psychomotor development during the first 3 years of life in Infants of type-1 Diabetic Mothers (IDMs).

Materials and methods: VEPs and psychomotor development were assessed serially between 2 months and 3 years of age in 16 IDMs (11 females, 5 males). Latency and amplitude of VEPs were compared with data obtained from a matched control group of 23 healthy children of non-diabetic mothers. VEP recording: 1. Binocular stimulation (white light, intensity 0,3 Joule, repetition rate 2 Hz). 2. Responses were recorded according to the American Electroencephalographic Society guidelines with a bandpass of 1-100 Hz. At least two trials of 100 artefact-free responses were recorded within 512 ms after stimulus. 3. Peak latencies and peak-to-peak amplitudes of all components were measured but only the most stable components (III, IV, V) were used for statistical comparisons. Psychomotor development test: Brunet-Lézine. Statistical analysis: ANOVA, non parametric tests and χ^2 with Yates correction when appropriate.

Results: All IDMs had normal outcome of global psychomotor development (IQ > 80). At the age of two months, IDMs showed mean latency of all VEP components significantly delayed compared with controls (right: latency of the forth component $199 \pm 32,8$ vs $155,6 \pm 29,0$ of controls [P 0,001]; left: latency of the forth component $194,7 \pm 35,74$ vs $155,3 \pm 30,3$ of controls [P 0,001]). At the end of follow-up, the latency of the forth (P2) component was still delayed compared with controls (right: $105,53 \pm 13,1$ vs $99,96 \pm 8,8$ of

controls [P 0,001]; left: $106,13 \pm 14,0$ vs $98,69 \pm 10,2$ of controls [P 0,001]). Abnormal VEPs (latency + 3 SD from normal mean value) were found in 2 cases (13%).

Conclusion: During the first 3 years of life, children of diabetic mothers had a normal outcome of global psychomotor development, but their VEPs showed a delayed maturation with mean P2 latency slower than controls, and abnormal responses in some cases. In these children, evoked potentials seem to be particularly sensitive tool to evidence subtle functional abnormalities of the central nervous system.

PS 111 Vascular function: cellular

1212

Glyoxalase I knock down in human aortic endothelial cells: a proteomic based approach

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Background and aims: Hyperglycemia plays an important role in the pathogenesis of diabetic complications including accumulation of methylglyoxal (MG), a highly reactive α -oxoaldehyde that is formed in cells primarily from the triose phosphate intermediates of glycolysis, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate and increased generation of advanced glycation endproducts (AGEs). The principal targets of MG modification are arginine residues of proteins. Reaction of MG with arginine in a non-enzymatic reaction leads to the formation of AGEs such as argpyrimidine and hydroimidazolone. Furthermore, MG is involved in regulation of different genes by modification of distinct transcription factors like mSin3A and Yap1. Glyoxalase 1 (GLO1), together with glyoxalase 2 and the co-factor glutathione, constitute the glyoxalase system, which is responsible for the detoxification of MG. A GLO1 specific knock down results in accumulation of MG in cells. **Methods:** The human aortic endothelial cells (HAEC) were transfected with GLO1-specific siRNA and cultured under hyperglycemic conditions (25 mM glucose). In order to achieve a better understanding of MG regulated gene expression 2D-DiGE proteomics technique was used to examine the changes in protein expression induced in HAEC by GLO1 knock down. Moreover, we investigated the effects of the knock down on apoptosis and oxidative stress by flow cytometry.

Results: GLO1-RNA was significantly downregulated about 90 % in these experiments if compared to siRNA-control. Proteomics analysis identified targets being significantly downregulated by intracellular MG accumulation, which can be grouped according to function: RNA stability and translation: HNRNPA1 (fold change (FC)=1.49) and EF1alpha (FC=1.43). Procollagen hydroxylation: LEPREL-2 (FC=1.40) and PLOD-2 (FC=1.43). Cytoskeleton and intermediate filaments: Vimentin (FC=2.11) and Lamin A/C (FC=1.74). There was a trend to down-regulation of GAPDH and malatdehydrogenase. Regarding oxidative stress there was no significant increase compared to the control transfection with non-specific, scrambled siRNA. Apoptosis was significantly increased under these conditions (161 %, compared to siRNA-control).

Conclusion: Several proteins have been identified as being regulated under hyperglycemic conditions in combination with a GLO1 knock down. The effect of MG accumulation is of concern regarding biosynthesis, posttranslational modification and structural stabilization. These modifications could at least explain the proapoptotic trend of the cells. Further evaluation and verification of these findings is needed to elucidate the effect of MG as a proapoptotic compound.

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1213

Endogenous hydrogen sulphide (H₂S) and novel slow release H₂S donors protect human microvascular endothelial cells from oxidative stress-induced cell death

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Background and aims: Significant microvascular endothelial dysfunction (MED) is observed in patients with type II diabetes mellitus (T2DM). However, the underlying mechanisms for this phenomenon are not fully understood but may involve oxidative / glycoxidative, increased production of cytotoxic lipid peroxides and glycation agents and mitochondrial toxicity. Recently we showed that lower levels of the endothelium-dependent endogenous gaseous vasodilator hydrogen sulfide (H₂S) in overweight and T2DM subjects compared to age-matched controls and that plasma H₂S levels were strongly negatively correlated with impaired microvascular function, insulin sensitivity and glycaemic control *in vivo*. H₂S is synthesised in humans from L-cysteine by the pyridoxal phosphate-dependent enzymes cystathionine-

beta-synthase (CBS) and cystathionine-gamma-lyase (CSE). Since H₂S is known to 'scavenge' oxidant species detrimental to the vasculature *in vitro* we aimed to determine whether endogenous H₂S and novel slow release H₂S donor molecules (SRHD) could prevent or reverse oxidative stress-induced MED.

Materials and methods: Human microvascular endothelial cells (HMEC) were exposed to oxidative stress inducing agents (SIN-1, a peroxynitrite donor), hydrogen peroxide, the lipid peroxidation product 4-hydroxynonenal (4-HNE) and the glycoxidation product methylglyoxal (MG). Cell viability was determined by alamar blue assay and mitochondrial membrane potential ($\Delta\Psi_m$) determined using tetramethylrhodamine methyl ester (TMRM). Endogenous H₂S synthesis was inhibited using D,L-propargylglycine (PAG; to inhibit CSE) and aminooxyacetate (AOAA; to inhibit CBS). Pharmacological H₂S was generated using GYY41317, MC0510 and MC0610; novel slow release donors which model CSE/CBS-derived H₂S synthesis.

Results: SIN-1 (20–500 μ M), 4-HNE (1–20 μ M) and MG (20–200 μ M) induced significant concentration-dependent reduction in cell viability (each $p < 0.01$, ANOVA post hoc Dunnett's test). Concentrations of SIN-1, 4-HNE and MG of 100 μ M, 10 μ M and 100 μ M were used for further studies. Incubation of cells with H₂S synthesis inhibitors PAG (1 mM) and AOAA (1 mM) for 1 hour prior to induction of oxidative stress significantly increased SIN-1, MG and 4-HNE induced cell death (each $p < 0.01$ c.f. oxidant treatment alone) and loss of mitochondrial $\Delta\Psi_m$. In sharp contrast, treatment of HMEC with SRHD (100–500 μ M) significantly preserved mitochondrial $\Delta\Psi_m$ and inhibited oxidant induced cell death.

Conclusion: Our study shows slow release H₂S donors can inhibit and reverse oxidative stress mediated cell injury. Strategies which increase vascular H₂S bioavailability may represent a novel therapeutic strategy to limit micro (and macro)vascular endothelial dysfunction in subjects with pre-diabetes and diabetes and maintain vascular health.

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1214

Oleic acid increases VEGF synthesis and secretion in aortic vascular smooth muscle cells via PI3-K and MAPK pathways: interplay with insulin and insulin resistance

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Background and aims: It is known that Free Fatty Acids (FFA) are not only energetic fuels, but also molecules able to induce insulin resistance by inhibiting the insulin signalling at different steps. They circulating concentrations are increased in obesity. The interplay among FFA, insulin and insulin-resistance in Vascular Smooth Muscle Cells (VSMC) are not fully clarified as yet. In other cell types, FFA increase the synthesis of Vascular Endothelial Growth Factor (VEGF), a molecule involved in mechanisms of vascular damage (such as instabilization of the atherosclerotic plaque) and of vascular repair (such as collateral vessel formation). Studies of our laboratory demonstrated that in VSMC insulin increases the synthesis of VEGF via PI3-K and MAPK signalling pathways, a phenomenon impaired in insulin resistant states. Aim of this study is to evaluate in VSMC: i) whether oleic acid induces synthesis/secretion of VEGF and interferes with the insulin action on the same phenomenon; ii) the signalling molecules involved; iii) whether these putative effects of oleic acid are modified by insulin resistance.

Materials and methods: The study has been carried out in VSMC obtained as primary culture in our laboratory from aortas of insulin sensitive lean Zucker rats and insulin resistant obese Zucker rats. We measured the influence of a 24-hour incubation with 50–100 microM oleic acid and/or with 2 nM insulin on VEGF protein synthesis and secretion (western immunoblotting and ELISA) (n=6). To evaluate the signalling molecules involved, the experiments were repeated after 60 min pre-incubation with: i) LY294002 (50 microM) and wortmannin (100 nM), specific PI3-K inhibitors; ii) rapamycin (100 nM), a specific inhibitor of mTOR, a molecule activated also by Akt; iii) specific inhibitors of molecules of the MAPK pathway: a) PD98059 (30 microM), inhibitor of ERK-1/2; b) SP600125 (15 microM), inhibitor of JNK-1/2; c) SB203580 (10 microM), inhibitor of p38 MAPK. Results are expressed as mean \pm SEM.

Results: In aortic VSMC from lean insulin sensitive Zucker rats: i) oleic acid 50–100 microM dose-dependently increased VEGF synthesis and secretion ($p=0.003$ – 0.0001); ii) oleic acid did not modify the ability of insulin to in-

crease VEGF synthesis and secretion. Furthermore, in these cells the effects of oleic acid on both synthesis and secretion of VEGF: a) are mediated by PI3K/Akt-pathway activation, being blunted by LY294002 and wortmannin ($p=0.04$ – 0.0001), but without an involvement of mTOR, being unaffected by rapamycin; b) are mediated by ERK-1/2 and JNK 1/2 activation, being blunted by PD98059 and SP600125 ($p=0.05$ – 0.027), but without an involvement of p38MAPK, being unaffected by SB203580. In aortic VSMC from obese Zucker rats oleic acid did not increase synthesis and secretion of VEGF.

Conclusion: Oleic acid increases VEGF synthesis and secretion in aortic VSMC from lean, insulin-sensitive Zucker rats with a mechanism mediated by molecules of PI3-K/Akt and of MAPK pathways, without interfering with the similar action exerted by insulin. These effects are lost in aortic VSMC from obese, insulin resistant Zucker rats, that are therefore resistant to both insulin and oleic acid as far as VEGF synthesis and secretion is concerned.

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1215

Chronic *in vivo* hyperglycaemia increases nitroxidant stress and hinders nitric oxide availability in human vessels and human endothelial cells

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Background and aims: Impaired endothelium-mediated vasodilation has been described in diabetic patients, suggesting decreased NO availability. However, a hyperglycaemia induced decrease in endothelial Nitric Oxide Synthase (eNOS) expression and activity is far from being firmly demonstrated. Indeed, hyperglycaemia increases superoxide anion (O_2^-) production: this might actually increase eNOS expression and activity, but divert Nitric Oxide (NO) to the formation of peroxynitrite ($ONOO^-$) and then nitrotyrosine (NT) within the vascular wall, thus inducing vascular damage and reducing NO bioavailability. Aim of the present study was to determine whether in the umbilical cord vessels and endothelial cells obtained from women with gestational diabetes (and thus with chronically elevated blood glucose levels) increased NT and eNOS levels were present and to investigate whether eNOS uncoupling and changes in NO bioavailability occur in these conditions.

Materials and methods: Tissue specimens and cultured human umbilical vein endothelial cells (HUVEC) were obtained from umbilical cords of 10 healthy women (Control, C) and 10 women with Gestational Diabetes (GD). Vascular NT and eNOS protein levels were evaluated in cord tissues (immunohistochemistry); NT and O_2^- levels (immunofluorescence), eNOS mRNA and protein (ratio of eNOS monomers to dimers) levels (Real-Time PCR and Western Blot), eNOS activity (conversion of [3H]-L-arginine in [3H]-L-citrulline) and cGMP production (ELISA) were measured in C- and GD-HUVEC.

Results: As compared to the proper controls, NT levels were significantly increased both in GD umbilical cords (25.90 ± 2.02 vs 11.70 ± 7.47 , $p < 0.05$ at MetaMorph Analysis System quantitation) and in GD-HUVEC (0.048 ± 0.003 vs 0.039 ± 0.003 AU, $p < 0.05$). As expected, GD cells showed markedly increased O_2^- production. eNOS expression resulted two-fold increased both in GD tissue specimens (39.04 ± 12.12 vs 17.29 ± 13.24 , $p < 0.003$) and in GD-HUVEC (eNOS/GAPDH mRNA = 1.0 ± 0.1 vs 0.6 ± 0.05 ; eNOS protein 0.64 ± 0.09 vs 0.37 ± 0.01 AU) but with a comparable ratio eNOS monomers/dimers in C and GD samples. Increased NOS activity was observed in GD-HUVEC (0.20 ± 0.02 vs 0.12 ± 0.03 pmol/mg protein⁻¹/min⁻¹, $p < 0.05$) which however was not paralleled by an increase in cGMP production, a natural target of NO activity (18.1 ± 4.24 vs 22.04 ± 5.61 fmol/ml).

Conclusion: Our data indicate that in presence of chronic hyperglycaemia endothelium from GD umbilical cords is characterized by increased NT production driven by increased O_2^- and NO generation. These data further indicate that hyperglycaemia, by increasing O_2^- levels, decreases vascular NO availability via NO consumption rather than through eNOS uncoupling, thus contributing to endothelial dysfunction in diabetes.

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Angiotensin II type 1 receptor, but not type 2, interferes with the insulin-induced nitric oxide production in HUVECs

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Background and aims: Angiotensin II (ATII) is a bioactive peptide of the renin-angiotensin-aldosterone system that plays an important role in the regulation of cardiovascular system and in the development of cardiovascular diseases. ATII exerts its different biological activities by binding to one of two known and well characterized receptor types, AT₁R and AT₂R. Despite the fact that these receptors share a similar high affinity to ATII, the consequences of their binding are opposite. AT₁R mediates ATII-induced vasoconstriction, cellular proliferation, inflammation and extracellular matrix remodelling. Conversely, AT₂R provides a protective role promoting vasodilatation, anti-proliferative and anti-inflammatory effects. We demonstrated that ATII impairs insulin signaling involved in nitric oxide (NO) production through IRS1/PI3K/Akt/eNOS pathway. In particular, the uncoupling of IRS1 and PI3K in ATII-treated endothelial cells may be linked to an increased phosphorylation at Ser³¹² and Ser⁶¹⁶ of IRS1, mediated by JNK and ERK 1/2, respectively. These changes are associated with a concomitant reduction in phosphorylation of Tyr⁶¹² and Tyr⁶³², resulting in an impaired activation of IRS1/Akt/eNOS pathway. All these effects occurred via the AT₁R stimulation, as deduced by the ability of the selective AT₁R antagonist, losartan, to revert the inhibitory effect of ATII. It has been proposed that the beneficial effects related to the use of AT₁R blockers is due also to the stimulation of AT₂R resulting from AT₁R blockage. The aim of this study was to investigate in HUVECs the effect of AT₂R blockage, evaluating whether the AT₂R is directly involved in the ATII effects on insulin signaling leading to NO production.

Materials and methods: HUVECs were incubated with ATII (100 nmol/l), AT₁R and AT₂R inhibitors (losartan 200nmol/l and PD123319 2mmol/l), alone or in combination, in the presence or absence of insulin (100 nmol/l). eNOS Activity Assay kit was used to measure eNOS activity.

Results: Exposure of HUVECs to ATII resulted in a time-dependent increase in IRS1 phosphorylation at both Ser³¹² and Ser⁶¹⁶, and in a parallel increase in phosphorylation of JNK and ERK1/2. Insulin increased by 2.7 fold ($p < 0.02$) the binding of IRS1 to the p85 subunit, while ATII treatment reduced this binding by 55%. ATII treatment resulted also in 55% inhibition of insulin-stimulated tyrosine phosphorylation of IRS1 in comparison with insulin alone ($p < 0.01$). Insulin increased by 3.5 fold ($p < 0.01$) the Ser⁴⁷³ Akt phosphorylation and by 2.6 fold ($p < 0.02$) the phosphorylation of eNOS on Ser¹¹⁷⁷, while ATII treatment reduced it by 56% and 62% ($p < 0.01$) respectively. eNOS activity was increased 2.5 fold ($p < 0.01$) by insulin, while it was inhibited by ATII ($p < 0.02$). Treatment of HUVECs with losartan reversed the inhibitory effects of ATII on insulin sensitivity, while no effects were observed with the PD123319. Furthermore, the simultaneous incubation with both inhibitors did not change the stimulatory effects of insulin as compared with losartan alone.

Conclusion: Our data extend the previous knowledge about the role of AT₁R and AT₂R on insulin signalling pathway. On the basis of these evidences, it is realistic to propose that the biological and protective effects typical of ATII receptor blockers are exclusively induced by the specific inhibition of AT₁R and not by the stimulation of AT₂R.

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Signal crosstalk between S1P and angiotensin II in human microvascular endothelial cells: participation of GAP proteins

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Background and aims: Sphingosine 1-phosphate (S1P) is a biologically active lipid generated by membrane lipids in activated platelets. S1P organizes actin into a strong cortical ring and strengthens both intercellular and cell-matrix adherens. S1P (500nM) induced small GTPase Rac activation and stimulated the phosphorylation of focal adhesion kinase (FAK) Y⁵⁷⁶, and FA (Focal Adhesion) proteins (FAK and paxillin) were linked to FA redistribution to the cell periphery. These effects are linked to S1P-induced endothelial barrier enhancement. Angiotensin II (AII) is a key molecule in the progression of

diabetic complications leading to endothelial dysfunction, cellular proliferation and inflammation. AII is known to induce actin-containing stress fiber formation and small GTPase Rho activation, involved in the process leading to endothelial barrier dysfunction. We have reported S1P-analog induced redistribution of FA proteins and regulation of Rac/Rho activity. Considering the opposite role of Rac/Rho in endothelial barrier regulation, S1P may ameliorate AII-induced endothelial barrier dysfunction, one of the important mechanisms underlying diabetic angiopathy. Small G proteins cycle between a GTP-bound “on” state and a GDP-bound “off” state are negatively regulated by GTPase-activating proteins (GAPs), which accelerate the small GTPase’s intrinsic hydrolysis of bound GTP to GDP. RhoGAPs thus act as an inhibitor of Rho activation. If there exists a signal crosstalk between S1P and AII, RhoGAPs may be involved in the regulation of small GTPase activity.

Materials and methods: Quiescent human microvascular endothelial cells (HMVECs) were stimulated with AII (100nM, 10min.) and / or S1P (30min.). 1) To monitor FA proteins and actin redistribution, immunofluorescent microscopies were performed. 2) To investigate the signal crosstalk between S1P and AII, Rac and Rho activity assay and RhoGAP activity assay was performed.

Results: 1) Immunofluorescent microscopy revealed that actin-containing stress fibers and FAs redistribution were observed under AII stimulated condition. On the other hand, the cells pre-treated with AII were mimicked actin and FA proteins redistribution by post-treatment with S1P as observed stimulation of S1P only. Contrary, AII reorganized the S1P-induced cortical distribution of actin and FA proteins into stress fibers and newly formed FAs, respectively. The distribution of AJ proteins also suggested counter effects between AII and S1P on AJ assembly. 2) Rac activity assay and Rho activity assay revealed S1P induced Rac activation and this activity returned to the basal level after AII treatment, whereas S1P restored AII-stimulated Rho activity to the basal level. RhoGAP assay revealed AII decreased RhoGAP activation. S1P stimulated RhoGAP activation in both AII-treated and non-treated cells. Elevated RhoGAP activity returned to the basal level by AII.

Conclusion: In this study, S1P revealed the protective effects against AII stimulated condition. S1P also restored elevated Rho activation by AII pre-treatment. S1P induced activation of RhoGAP. Our results strongly suggest signal crosstalk between S1P and AII in the regulation of small GTPase activity. RhoGAPs may at least partially participate in this process involving in endothelial barrier regulation, suggesting the possible role of S1P to ameliorate AII-induced vascular disorder.

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SLC24A6 is involved in high glucose-induced endothelial cell apoptosis via regulating intracellular calcium concentration

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Background and aims: Endothelial dysfunction contributes to the pathogenesis of vascular diseases in diabetics. Several lines of evidence has indicated that high glucose-induced apoptosis of endothelial cells plays important roles in the pathogenesis of endothelial dysfunction. However, the underlying mechanisms by which high glucose initiates apoptosis in endothelial cells have not been fully elucidated. In the present study, we investigated the roles and possible mechanisms of a Na⁺/Ca²⁺ exchanger, SLC24A6, in high glucose-induced apoptosis in endothelial cells.

Materials and methods: Lentivirus containing full-length cDNA of human SLC24A6 (Lenti-SLC24A6) and small interfering RNA designed for human SLC24A6 (SLC24A6 siRNA) were generated. After human aortic endothelial cells (HAEC) were transfected with Lenti-SLC24A6 or SLC24A6 siRNA, cell apoptosis was evaluated by DNA fragmentation analysis, the intracellular Ca²⁺ concentration ([Ca²⁺]_i) of HAEC was determined by using Fura-2 ratio technique, calcineurin phosphatase activity was measured using Calcineurin Cellular Activity Assay Kit, and phosphorylated BAD (phospho-BAD) was investigated by western blotting. HAEC were cultured with 30 mmol/L (high glucose) or 5.6 mmol/L glucose (low glucose) for 96 h, and then SLC24A6 expression was detected by western blotting. Cell apoptosis, [Ca²⁺]_i, calcineurin phosphatase activity, and phospho-BAD were also investigated in glucose-treated HAEC. Meanwhile, the impacts of high glucose on cell apoptosis were evaluated after HAEC were transfected with SLC24A6 siRNA or treated with FK506, an inhibitor of calcineurin. Furthermore, we investigated SLC24A6 expression, calcineurin phosphatase activity, and phospho-BAD in aorta tissues of the diabetics, STZ-induced diabetic monkeys, and db/db mice.

Results: Lenti-SLC24A6 transfection induced a significant enhancement in apoptotic cells. The overexpression of SLC24A6 in HEAC caused a significant increase in [Ca²⁺]_i, with significantly increased calcineurin phosphatase activity and decreased phospho-BAD. When compared with low-glucose culture for 96 h, high-glucose culture resulted in a 3.2-fold increase in apoptotic cells, a 5-fold increase in SLC24A6 levels, a 2.2-fold increase in calcineurin phosphatase activity, and a 3.4-fold decrease in phospho-BAD in HAEC. Both siRNA-mediated knockdown of SLC24A6 and treatment with FK506 significantly lowered the number of apoptotic cells induced by high glucose. In aorta tissues, it was found that both SLC24A6 levels and calcineurin phosphatase activities significantly increased in the diabetics, STZ-induced diabetic monkeys, and db/db mice when compared with their corresponding controls. However, phospho-BAD was detected with significantly decreased levels in these diabetic aorta tissues.

Conclusion: The current data demonstrated that SLC24A6 maybe play an important role in high glucose-induced apoptosis of aortic endothelial cells. We also presented evidence that SLC24A6 was involved in regulation of intracellular Ca²⁺ concentration, which then affected calcineurin phosphatase activity and phosphorylated level of BAD. The study suggested that SLC24A6-mediated Ca²⁺/calcineurin pathway could be a novel component in the pathogenesis of diabetic macrovascular complication.

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MiR-216a regulates autophagy during vascular senescence

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Background and aims: Aging is one of the major risk factors for type 2 diabetes mellitus and atherosclerosis. Among the age associated functional and structural changes, of particular note is the decline in endothelial function. Autophagy, an intracellular catabolic process involved in protein and organelle degradation via the lysosomal pathway, declines with aging leading to the accumulation of intracellular waste products, and there is a clear overlap between the signaling networks regulating both aging and autophagy. The role of autophagy in diabetic vascular disease is poorly understood, but basal autophagy is considered a survival mechanism safeguarding cells against cellular distress, in particular oxidative injury, metabolic stress and inflammation, by removing harmful modified proteins and damaged components. The importance of the delicate and coordinated regulation of autophagic responses during metabolic homeostasis is clear, and small-molecule modulators of autophagy or central molecules controlling this process could facilitate the exploitation of this adaptive pathway for therapeutic interventions against metabolic and inflammatory diseases. Among modulators, microRNAs may represent good candidates for regulating autophagy.

Materials and methods: Computational analysis reveals that crucial autophagy related genes, such as Beclin1 and Atg5 are potential targets of miR-216a, a microRNA that is highly expressed as a consequence of diabetes. First, we compared the miR-216a expression in old HUVECs (PDL44) versus young cells (PDL8) and in the presence of high glucose levels (25 mM). Then we performed transfection experiments in HUVECs in order to modulate miR-216a expression. Finally we investigate the role of miR-216a *in vivo* in atherosclerotic plaques.

Results: Our results show a significant upregulation of miR-216a expression during aging and glucotoxicity, suggesting a downstream effect of post-transcriptional suppression of target genes, leading to the signalling abnormalities in the aging phenotypes. Moreover, in the same conditions, expression of Beclin1 and Atg5 was correspondingly downregulated, as assessed by RT-PCR and Western blot analysis. Transfection of PDL44 endothelial cells with an oligonucleotide that decreases miR-216a level (AS216a) permits an increase in Beclin1 and Atg5 expression, both at mRNA and protein levels. Conversely, overexpression of the miR-216a precursor decreases Beclin1 and Atg5 mRNA and protein expression levels in PDL8 cells. Preliminary analysis indicates that overexpression of miR-216a in young cells results also in a mRNA reduction of a putative target gene TP53INP1. Because autophagy plays a central role in atherosclerotic plaque stabilization we examined miR-216a, Beclin1 and Atg5 expression in plaques from patients who underwent carotid endarterectomy for symptomatic disease, confirming a negative correlation between miR-216a and autophagic genes mRNA level in an *in vivo* setting (R = -0.4, p<0.005). Furthermore, miR-216a expression in atherosclerotic plaque was correlated with HOMA-IR (R = 0.32; p<0.05).

Conclusion: Our data suggest that vascular senescence is associated to decreased expression of autophagy genes as a consequence of increased miR-216a expression, a microRNA particularly overexpressed in diabetic vascular disease.

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Impact of pancreatic endothelium on secretory capacity and proliferation of pancreatic islet cells

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Background and aims: The pancreatic islets are one of the most vascularised organs of the body. Beta-cells are not directly exposed to the blood flow but are separated from the blood by a thin layer of endothelial cells (EC). The close proximity of EC and beta-cells suggests that there is a cross-talk relationship between these both cell types. The main objective of this work is to investigate if pancreatic endothelium could affect islet cell function and growth.

Materials and methods: Rat pancreatic islets were isolated using density gradient and then, were disaggregated with trypsin treatment or used to isolate EC. For the pancreatic EC extraction, islets were placed onto collagen gel and EC grown on this base. After one week, EC were trypsinized and were maintained in culture until passage number 8-10. The purified EC were identified by immunohistochemistry for expression of von Willebrand factor (vWF) and CD31. Islet disaggregated cells were cultured in the presence or absence of EC. Islet disaggregated cells were characterised by immunohistochemistry for expression of insulin and glucagon. To investigate the impact of the pancreatic endothelium on beta-cell function, we measured insulin secretion in response to glucose. Growth capacity of islet cells was determined by staining of the proliferation marker, Ki67.

Results: Immunofluorescence staining showed that islets EC expressed vWF and CD31. In disaggregated islets, the insulin secretion in response to 16.7mM glucose concentration was increased of 3.4 fold compared to 5.5mM glucose concentration. Interestingly, disaggregated islets cultured during three days with EC showed greater insulin secretion capacity in response to the glucose (5.1 fold) than single disaggregated islets. This increase was more important after six days of co-culture (9.4 fold). The immunofluorescence staining for pancreatic hormones showed well-done disaggregation of islets with the presence of single cells with expression of glucagon or insulin. Nevertheless, in the presence of EC, the disaggregated islets seem to reorganise in cell clusters showing insulin but also glucagon expression. Moreover, the presence of EC allowed the proliferation of islet cells which was demonstrated by Ki67 immunolabelling in the nucleus of glucagon and insulin-marked cells.

Conclusion: We showed that EC basement promotes the capacity of insulin secretion in response to glucose and the proliferation of islet cells.

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Even mild hyperglycaemia disturbs vascular homeostasis in humans

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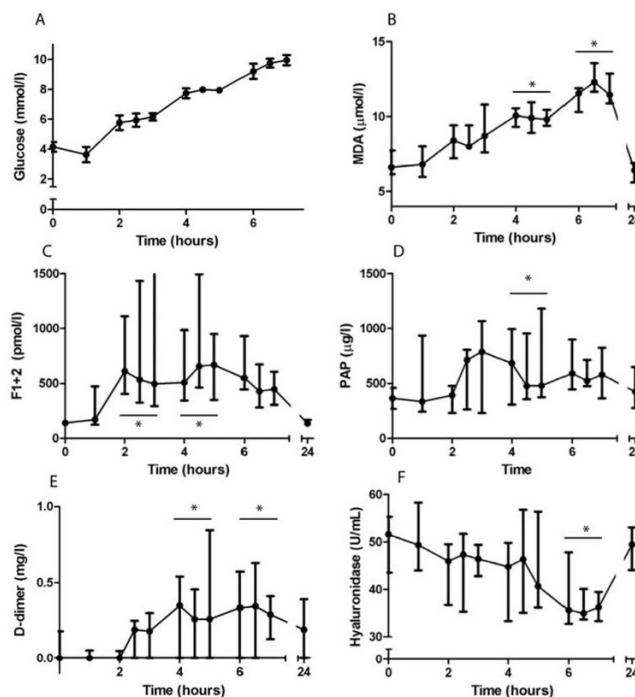
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Background and aims: Hyperglycemia induces oxidative stress, damage to the endothelial glycocalyx and a prothrombotic shift in coagulation and fibrinolysis. These factors are known contributors to vascular disease but it is unknown at what glucose level these changes occur. The aim of this study was therefore to determine the level of glycemia at which these changes start.

Materials and methods: In 11 healthy young males we performed a stepwise normo-insulinemic hyperglycemic clamp at plasma glucose (PG) levels of 6, 8 and 10 mmol/l for two hours each (fig A). Octreotide was infused to suppress endogenous insulin release. Measurements were performed at baseline, after a 1-hr octreotide run-in, every 30 minutes during the clamp and at 24-hrs. Oxidative stress was assessed by determining malondialdehyde (MDA) using tandem mass spectrometry; coagulation activation by von Willebrand factor (vWf, Elisa) and prothrombin fragment 1+2 (F1+2, Elisa); fibrinolysis by plasmin-alpha2-antiplasmin complexes (PAP, Elisa) and d-dimer (BCS-XP); and glycocalyx turnover by hyaluronic acid (HA) and hyaluronidase (both Elisa). Differences between plateaus were assessed by a Wilcoxon signed ranks test for paired data. The influence of time on the measurements at each glucose level was assessed using the Friedman test.

Results: MDA showed a gradual increase highly correlating with PG ($p=0.82$, $p<0.001$, fig B). F1+2 significantly increased at 6 mmol/l, maintaining the same level at all PG concentrations (fig C) and both PAP and d-dimer increased significantly at 8 mmol/l, indicating coagulation activation followed by fibrinolysis (fig D&E). vWf showed a small, but significant decrease at all PG levels (max 9%, $p=0.01$). No difference in HA levels was detected, however hyaluronidase showed a gradual decrease, significant at 10 mmol/l (fig F), indicating increased hyaluronidase substrate binding. There was no indication of a cumulative effect of PG on any of the parameters.

Conclusion: Our results show that glucose-induced changes to vascular homeostasis already start at near normal glucose levels. Furthermore our study reveals a dose-dependent effect of glucose on MDA formation and an on-off phenomenon for glucose induced coagulation activation, while changes to the endothelial glycocalyx occur at glucose levels of 10 mmol/l or higher. These results give us more insight in the glucose driven mechanisms of vascular complications in humans.



* $p<0.05$ compared to $t=1$, after 1-hr octreotide infusion
Glucose values are expressed as mean [SD], outcome parameters as median [IQR]

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Oxygen increases arterial stiffness and blood pressure in patients with type 1 diabetesD. Gordin¹, L. Bernardi², M. Rosengård-Bärlund¹, V.-P. Mäkinen¹, A. Soro-Paavonen¹, C. Forsblom¹, P.-H. Groop¹;¹Medicine, Division of Nephrology, Folkhälsan, Institute of genetics, Folkhälsan research center, Helsinki, Finland, ²Department of Internal Medicine, University of Pavia and IRCCS Policlinico S. Matteo, Pavia, Italy.

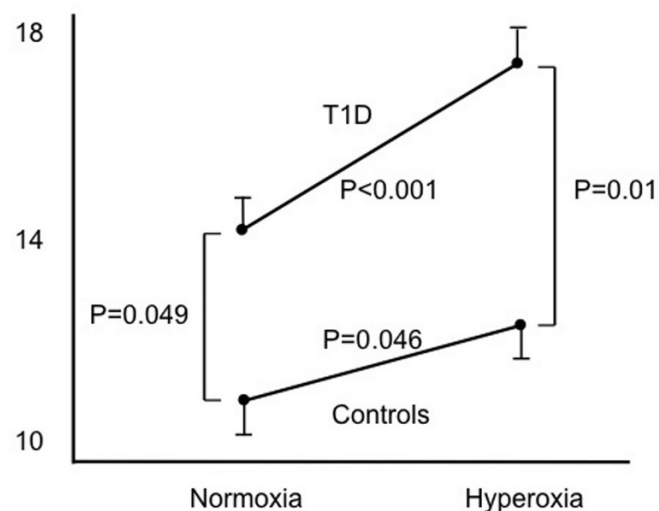
Background and aims: Oxygen is often empirically used in the treatment of different medical conditions. However, hyperoxia has in a few recent studies been shown to increase systemic arterial stiffness in healthy volunteers. Although arterial stiffness is increased in patients with type 1 diabetes (T1D), no studies have yet explored systemic arterial stiffness in response to hyperoxia in this patient group. Therefore, we examined the effect of short-term oxygen administration on arterial stiffness, blood pressure, and heart rate in a large number of patients with T1D and healthy subjects.

Materials and methods: Altogether 98 patients with T1D and 49 control subjects were studied. Continuous finger pressure waveforms were monitored with a digital plethysmograph (Finapres) both at steady state (10 min) and during hyperoxia (15 min). The augmentation index (AIx) was calculated from the waveforms and used as an estimate of arterial stiffness. In order to validate the method (estimate central aortic stiffness) we calculated a transfer function after measuring pressure waveforms simultaneously from the radial artery (applanation tonometry, Sphygmocor) and the finger (Finapres) in 15 subjects.

Results: The groups did not differ with regard to age (31.9 ± 0.7 [T1D] vs. 32.7 ± 1.3 [Controls], $P = \text{NS}$). AIx increased from 14.1 ± 0.9 to $17.6 \pm 0.8\%$, ($P < 0.001$) in patients with T1D and from 10.7 ± 1.5 to 12.6 ± 1.3 ($P < 0.05$) in controls in response to hyperoxia (Figure). However, the increase in AIx (3.4 ± 0.5 vs. $1.5 \pm 0.7\%$, $P = 0.03$), systolic (6.7 ± 1.2 vs. 1.2 ± 2.0 mmHg, $P = 0.02$) and diastolic 3.6 ± 0.7 vs. -0.2 ± 1.1 mmHg, $P = 0.004$) blood pressure was more pronounced in patients with T1D compared to controls. Oxygen saturation was lower in patients with T1D ($97.2 \pm 0.1\%$) than in healthy controls at baseline ($97.7 \pm 0.1\%$, $P < 0.01$) and increased more in response to hyperoxia (1.5 ± 0.1 vs. $1.1 \pm 0.1\%$, $P = 0.03$).

Conclusion: Arterial stiffness and blood pressure increased more in response to hyperoxia in patients with T1D than in healthy subjects, suggesting that oxygen should be administered cautiously in such patients. The rapid changes rather indicate a functional mechanism than structural modifications of the arterial wall.

AIx (%)



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Elevated frequencies of CD34+VE-cadherin+ endothelial progenitor cells (EPCs) and negative correlation between endothelial dysfunction and EPCs in children with type 1 diabetes on insulin pump therapyB. Głowińska-Olszewska¹, M. Moniuszko², A. Hryniewicz¹, M. Jeznach², M. Dąbrowska³, W. Łuczyński¹, A. Bodzenta-Łukaszyk², A. Bossowski¹;¹Department of Pediatrics, Endocrinology, Diabetology with Cardiology Division, Medical University, ²Department of Allergy and Internal Medicine, Medical University, ³Department of Hematological Diagnostics, Medical University, Białystok, Poland.

Background and aims: Endothelial progenitor cells (EPCs) are bone marrow derived pluripotent vascular progenitor cells capable to contribute to reendothelialization and neovascularization. The low number of circulating EPCs has been established as a biomarker of cardiovascular risk, and is known to be reduced in adults with atherosclerosis risk factors. In healthy children EPCs number is higher than in adults, but data on young populations with risk factors are limited. The aim of the study was to assess vascular function and structure together with EPCs in children with type 1 diabetes and to establish correlations between studied parameters.

Materials and methods: We compared 31 children and adolescents with diabetes type 1 (aged mean 14.4 yrs, diabetes duration mean: 5.7 yrs, HbA_{1c} level: 8.8%, all treated with insulin pumps) with 17 healthy age and gender matched control children. EPCs were analysed with use of the multicolor flow cytometry with combination of monoclonal antibodies against CD34, CD144 (VE-cadherin) and CD 309 (VEGFR-2). sICAM-1, hsCRP, thrombomodulin, adiponectin levels were assessed by ELISA methods. Vascular function and structure were evaluated by flow-mediated dilation (FMD) and carotid intima-media thickness (IMT) ultrasonographically. Data are presented as mean \pm SD, median or frequencies. T-student and median test were used for comparison between two groups, for correlations Pearson's analysis was performed.

Results: CD34+, and CD34+VEGFR-2+ EPC frequencies were similar in both groups ($P = 0.71$, $P = 0.8$ respectively). CD34+VE-cadherin+ and VE-cadherin+ frequencies were higher in diabetic children compared to healthy group ($P = 0.008$, $P = 0.039$ respectively). FMD was lower (9.3% vs 12.3% , $P = 0.03$), and IMT was higher (0.53 vs 0.45 mm, $P = 0.01$) in diabetic children. hsCRP, sICAM-1 and cholesterol levels were higher in the study group. There was a significant relationship between CD34+VEGFR-2+ and BMI ($r = 0.35$, $P = 0.04$), HDL ($r = -0.3$, $P = 0.04$), sICAM-1 ($r = 0.47$, $P = 0.019$), and FMD ($r = -0.54$, $P = 0.002$). CD34+VE-cadherin+ correlated with BMI ($r = 0.4$, $P = 0.04$) and FMD ($r = -0.6$, $P = 0.031$). HbA_{1c} level was not related to EPC counts.

Conclusion: Abnormalities of endothelial function and structure are present in poorly metabolically controlled children with diabetes type 1. Contrary to adult population with diabetes, diabetic children demonstrated unchanged (CD34+VEGFR2+) or even increased (CD34+VE-cadherin+) frequencies of EPC, that correlated negatively with endothelial function. This may be result of the effective mobilization of endothelial progenitor cells in young population at increased risk for atherosclerosis in order to repair damaged endothelium.

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Determinants of circulating endothelial progenitor cell number in prediabetic subjectsA. Angelidi¹, J. Protosaltis¹, A. Gritzapis¹, I. Kornezos², T. Sergeantanis¹, K. Tzirogiannis¹, E. Boutati³, G. Dimitriadis³, A. Melidonis¹, S. Raptis³;¹Diabetes Center, Tzanio General Hospital, Piraeus, ²Department of Radiology, Tzanio General Hospital, Piraeus, ³Hellenic National Diabetes Center & Attikon University Hospital, Athens, Greece.

Background and aims: Endothelial Progenitor Cells (EPCs) originate primarily from the bone marrow and take part in postnatal neovascularization. Moreover, when incorporate into endothelial damaged areas, they replace the dysfunctional vascular endothelium which predisposes to atherosclerosis. On the contrary, reduced EPCs are found to be associated with increased cardiovascular risk. The aim of this study was to investigate the relation of EPC numbers with several cardiovascular risk factors among patients with prediabetes.

Materials and methods: A total of 39 volunteers without history of cardiovascular disease were recruited and underwent a 75gr OGTT in order to confirm the presence of prediabetes. We recorded for all the study population: waist circumference, waist to hip ratio, body mass index, smoking habit,

presence of metabolic syndrome (NCEP-ATP III & IDF criteria) and homeostasis model assessment of the insulin resistance (HOMA-IR). Additionally, we measured all the well-established biomarkers of cardiovascular risk and high sensitivity C-reactive protein (hs-CRP). Visceral fat mass was ultrasonographically measured using a strict protocol suggested from previous studies. Flow cytometry identified and quantified the EPCs (CD34+ CD133+ KDR+ cells). Stepwise multivariate regression analysis was performed to determine the possible correlations of EPCs to the above mentioned risk factors.

Results: Univariate regression analyses performed separately for all cardiometabolic parameters: EPC counts were not significantly associated with the presence of metabolic syndrome or the cumulative number of its components. Conversely EPC numbers were significantly correlated with visceral fat ($\rho = -0.34$, $p = 0.040$), median arterial pressure ($\rho = -0.44$, $p = 0.009$), HOMA-IR ($\rho = -0.40$, $p = 0.031$), hs-CRP ($\rho = -0.44$, $p = 0.006$), waist circumference ($\rho = -0.41$, $p = 0.017$) and total cholesterol ($\rho = 0.47$, $p = 0.003$). However in multivariate regression analysis visceral fat only remained significantly associated with the number of EPCs (OR=0.79, 95%CI:0.64–0.98, $p = 0.032$).

Conclusion: Circulating endothelial progenitor cells in prediabetic subjects are negatively and independently correlated to visceral fat mass, which supports the importance of lifestyle modifications - and predominantly weight reduction - to prevent and minimize the progress of cardiovascular disease.

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Endothelial but not smooth muscle progenitor cells are reduced in type 2 diabetic patients independent of macrovascular disease

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Background and aims: Individuals with type 2 diabetes mellitus (T2DM) have increased rates of macrovascular disease (MVD), including coronary artery disease (CAD) and peripheral artery disease (PAD). Increasing evidence indicates involvement of aberrant circulating vascular progenitor cell frequency and function in the development of MVD. Endothelial Progenitor Cells (EPCs) are involved in maintaining endothelial integrity and can be considered a mirror of vascular repair capacity. On the other hand, Smooth Muscle Progenitor Cells (SMPCs) may promote luminal narrowing and aggravate development of MVD. However, the relationship between EPCs and SMPCs, and the development of accelerated MVD in T2DM is still unknown. We therefore assessed vascular progenitor cell frequency in T2DM patients and non-diabetic subjects with or without MVD.

Materials and methods: Peripheral blood was obtained from T2DM patients and non-diabetic subjects with or without documented CAD or PAD. The following groups were included: group 1: T2DM, no PAD/CAD ($n = 25$), group 2: T2DM, with CAD ($n = 14$), group 3: T2DM, with PAD ($n = 21$), group 4: healthy age-matched controls ($n = 18$), group 5: no T2DM, with CAD ($n = 8$), group 6: no T2DM, with PAD ($n = 21$). Circulating vascular progenitor cells were analyzed by flowcytometry using antibody cocktails against CD34/KDR/CD133 (EPCs), and CD14/CD105 (SMPCs).

Results: The number of CD34+ EPCs was reduced by 35% in T2DM patients with or without MVD compared with non-diabetic subjects [with or without MVD] (260 ± 15 vs. 353 ± 23 CD34+ cells/ 10^6 events respectively, $p < 0.001$). When comparing healthy control subjects with T2DM patients [with or without MVD], a 2.8-fold reduction in CD34+KDR+ EPCs was found (14 ± 4 vs. 5 ± 1 CD34+KDR+ cells/ 10^6 events, respectively, $p < 0.01$). However, a significant decrease ($p < 0.05$) in the number of CD34+KDR+ EPCs in non-diabetic subjects with CAD or PAD (4 ± 1 and 6 ± 1 CD34+KDR+ cells/ 10^6 events, respectively) compared with healthy control subjects (14 ± 4 CD34+KDR+ cells/ 10^6 events) was also observed. In contrast to EPCs, the frequency of circulating CD14+CD105+ SMPCs was not affected by T2DM compared with non-diabetic subjects. However, in non-diabetic patients the presence of PAD, but not CAD, was associated with an increase in SMPC frequency compared with healthy control subjects (3.2-fold increase; 3280 ± 494 vs. 1020 ± 332 CD14+CD105+ cells/ 10^6 events, $p < 0.01$). Diabetic patients with MVD had similar numbers of circulating SMPCs as diabetic patients without MVD.

Conclusion: The number of circulating CD34+ EPCs is significantly reduced in T2DM patients compared with non-diabetic subjects, independent of the presence or absence of MVD. Compared with healthy controls, the number of CD34+KDR+ EPCs is significantly decreased in both diabetic subjects,

and non-diabetic subjects with MVD. However, circulating SMPC numbers are not affected by T2DM. Therefore, an altered balance between circulating EPCs and SMPCs in favour of SMPCs may contribute to reduced vascular repair capacity and subsequent increased rates of MVD in T2DM.

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Close relationship between serum hyaluronan levels and vascular smooth muscle function in patients with type 2 diabetes

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Background and aims: Since cardiovascular disease is the major cause of mortality in patients with type 2 diabetes, a surrogate marker for vascular function is urgently needed. Such a marker would allow initiation of early intervention. Currently, endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent nitroglycerine-mediated dilation (NMD) are considered to be sensitive markers of vascular function. However, their clinical applications are limited because of technical and methodological problems such as nitroglycerin usage. On the other hand, hyaluronan is produced in the process of the tissue restoration after inflammation. Previous reports have indicated that the serum hyaluronan level is increased in patients with type 2 diabetes and is associated with metabolic markers including glycosylated hemoglobin (HbA1c) and body mass index (BMI). However, no study has as yet examined the relationship between hyaluronan and vascular function. Thus, we aimed to clarify whether hyaluronan can serve as a surrogate marker for vascular function.

Materials and methods: This study included 79 patients with type 2 diabetes (age, 62 ± 13 ; duration, 10.7 ± 9.0 years). Serum hyaluronan level in blood samples was measured using a latex immunoturbidimetric assay. Estimated glomerular filtration rate (eGFR) was calculated employing age, sex and serum creatinine concentrations. FMD and NMD were measured at the brachial artery using UNEXEF18G and expressed as changes in diameter. To evaluate the relationships of FMD and NMD to the serum hyaluronan level, uni- and multivariate regression analyses were performed.

Results: The mean (\pm SD) FMD and NMD were 4.4 ± 3.0 and $13.3 \pm 6.4\%$, respectively. In univariate regression analysis, FMD was significantly associated with age ($r = -0.490$, $p < 0.001$), diabetic duration ($r = -0.421$, $p = 0.001$), BMI ($r = 0.224$, $p = 0.030$), systolic blood pressure ($r = -0.240$, $p = 0.036$), eGFR ($r = 0.334$, $p = 0.002$) and hyaluronan ($r = -0.320$, $p = 0.004$). NMD was significantly associated with age ($r = -0.417$, $p < 0.001$), eGFR ($r = 0.248$, $p = 0.032$) and hyaluronan ($r = -0.392$, $p < 0.001$). In multivariate regression analysis, FMD was associated with age ($r = -0.399$, $p = 0.027$) and diabetic duration ($r = -0.276$, $p = 0.027$), whereas NMD was associated only with hyaluronan ($r = -0.401$, $p = 0.003$), suggesting elevated serum hyaluronan to possibly be responsible for dysfunction of vascular smooth muscle rather than the vascular endothelium.

Conclusion: The present study indicates that close relationship between hyaluronan levels and vascular smooth muscle function exists in patients with type 2 diabetes. Hyaluronan is a potential surrogate marker for vascular function in patients with type 2 diabetes, which is independent of other metabolic markers, although further study is necessary.

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Vasoprotective effects of caloric restriction in aging and type 2 diabetes

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Background and aims: Caloric restriction (CR) without malnutrition extends life span in a range of organisms including insects and mammals and lowers free radical production by the mitochondria. The direct effects of CR on vascular function and phenotype in aging are not well characterized. It is generally accepted that tonic release of nitric oxide (NO) from the endothelium exerts vasculoprotective and cardioprotective effects. The current study was undertaken to examine the effects of caloric restriction on endothelial

function, metabolic, glycation and oxidative stress markers, in old age Wistar and diabetic Goto-kakizaki (GK) rats.

Materials and methods: Wistar (W) and GK rats with twelve months old were progressively subject to CR up to 35% during 4 months and compared with ad libitum fed rats. The effects of CR were investigated on NO-dependent and independent vasorelaxation in isolated rat aortic arteries from the different groups. Metabolic profile, NO bioavailability, the accumulation of advanced glycation end-products (AGEs) and carboxymethyllysine (CML), the expression of the receptor for AGEs (RAGE) in the aorta and vascular oxidative stress were also evaluated.

Results: We have previously shown that aging was associated with increased endothelial dysfunction in both W and diabetic GK rats. In this work we show that GK rats exhibited a three-fold increment in vascular oxidative stress and in CML accumulation when compared with age-matched W rats. Diabetes-induced excess accumulation of reactive oxygen species in aorta resulted in vascular nitric oxide (NO)/cGMP dysfunction (decrement of 40 %, $p < 0.001$). Caloric restriction was able to retard the progression of endothelial dysfunction and significantly reduce aortic oxidative stress ($p < 0.001$) in W and diabetic GK rats. Furthermore, CML accumulation in aortic wall was sharply reduced and accompanied by a decrement in the expression of aortic RAGE in caloric-restricted fed animals. Noteworthy, the NO-cGMP pathway in the aorta of GK rats was partially reverted explaining the positive outcomes observed in the endothelial function of diabetic GK rats.

Conclusion: This study provides, to our knowledge, the first evidence that caloric restriction increases bioavailability of NO improving endothelial function by a mechanism that likely includes upregulation of eNOS and a decrement in glycation and oxidative biomarkers in aged type 2 diabetic rats. *Supported by: Faculty of Medicine, University of Coimbra*

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Fluctuating hyperglycaemia induces higher levels of oxidative stress than sustained hyperglycaemia in insulin resistant rats

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Background and aims: Recent reports have shown that despite similar mean glycaemic exposure measured as HbA1c levels, the risks of developing macro-vascular complications can be different in diabetic patients. Repeated episodes of short term hyperglycaemia not necessarily resulting in altered HbA1c levels have been hypothesized to promote the production of reactive oxygen species (ROS) more than sustained hyperglycaemia and thus lead to increased levels of oxidative stress. In the present study, we compare effects of fluctuating with sustained high or low levels of glucose on oxidative stress markers.

Materials and methods: A total of 30 catheterized diet induced obese (DIO) insulin resistant male Sprague-Dawley rats (38 weeks) were randomized into four weight-matched groups ($n = 7-8$) and connected to an automated blood sampling system (Accusampler[®]) for blood withdrawal and intravenous glucose infusions. Animals were fasted for 6 hours prior to receiving either a continuously high (CHG) or low (CLG) or pulsating (FLU) infusion of glucose for 96 hours. The FLU group received nine daily 30 min glucose infusions separated by 2.5 h. In total FLU and CLG groups received equal amounts of glucose whereas the CHG group received three times this amount. Plasma glucose and insulin was monitored during the complete study period. Plasma malondialdehyde (MDA), ascorbate (ASC) and dehydro-ascorbate (DHA), was measured every 24th hours. Plasma MDA was used as a biomarker of oxidative damage to lipids whereas plasma ASC and DHA were used to evaluate the antioxidant status by calculating the ascorbate oxidation ratio as % DHA of total ASC.

Results: All groups had similar basal plasma glucose (~ 6.5 mmol/l) and insulin (~ 600 pmol/l) levels. Hyperglycaemia (> 25 mmol/l) and hyperinsulinemia (> 2700 pmol/l) were immediately manifested in the CHG group and maintained throughout the study. The FLU group showed regular oscillation of glucose $\sim 20-22$ mmol/l and insulin ~ 2500 pmol/l at peak level and returned back to normal levels in between pulses. The CLG group displayed an immediate increase in glucose ~ 12 mmol/l but levelled out during the infusion period and was comparable to controls at the end. In turn - plasma insulin levels were gradually increased during infusion to levels of ~ 2000 pmol/l. Plasma MDA were significantly increased in the FLU group at 72 and 96 hours (72 hours: VEH: 1.42 ± 0.15 vs. FLU: 2.3 ± 0.15 ; 72 hours: VEH: 1.34 ± 0.15 vs. FLU: 2.15 ± 0.15 ; $p < 0.05$), whereas the CHG group only showed significance at 72 hours (VEH: 1.42 ± 0.15 vs. CHG: 2.35 ± 0.16 ; $p < 0.05$). Plasma ASC and %DHA were not different among groups ($p = ns$; all cases).

Conclusion: We show that fluctuating glucose levels lead to oxidative stress similarly to that of sustained hyperglycaemia despite a much lower total glycaemic exposure. Thus our data supports the notion that fluctuating glucose may be relatively more deleterious than sustained hyperglycaemia.

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Increasingly expressed sulfonylurea-regulated, non-selective cation channel mediates myocardial dysfunction and arrhythmias of obese rats after ischaemic injury

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Several studies have discovered that the SUR1-regulated nonspecific cation (NC_{Ca-ATP}) but not K_{ATP} channels are upregulated in ischemic astrocytes, and block of SUR1 with sulfonylurea (SU) reduced cerebral edema, infarct volume and mortality. Recent patch-clamp studies also found similar SUR-1 regulated non-selective cation channel (NSC_{Ca}) in myocardial cells, but the effects of blocking NSC_{Ca} by SUs on ischemic myocardium have not been reported. The aim of the study was to identify the up-regulation of NSC_{Ca} in myocardial cells after ischemic injury, and to evaluate the effects of gliclazide on myocardial infarct size, post-ischemic cardiac function and arrhythmia in obese rats with

cardiac ischemia-reperfusion (I/R) injury. Diet-induced obese rats divided into 4 groups. MI group rats received subcutaneous Isoprenaline (100mg/kg) shots to create myocardial I/R injury, and non-MI group rats received subcutaneous saline shots. These rats were sacrificed 24 hours later and had their hearts taken and prepared for immunofluorescence imaging. SU group and Control group rats were given gliclazide (1 mg/kg) or saline lavage Q12h for 48 hours. These rats were subjected to myocardial I/R injury by Isoprenaline (100mg/kg) injection subcutaneously 24 hours after first lavage. ECG was recorded and all the arrhythmias were analyzed with arrhythmia severity index (ASI). At the end of 24 hours after I/R injury, hemodynamic parameters were measured with an electromanometer. The hearts were harvested and cut into 1mm slides. The infarct-size was evaluated with TTC staining, calculated with Image-Pro Plus 6.0. Obvious up-regulation of SUR-1 was observed at the vascular wall of the heart in MI group, but not in non-MI group. For rats in the SU group and control group, blood glucose was only slightly declined during study. The mortality was 63.64% (14/22) in control group and 45.45% (10/22) in SU group (NS). The frequency of serious arrhythmia was lower in SU group (ASI: 2.14 ± 1.83 vs. 4.00 ± 2.85 , $p < 0.05$). Gliclazide administration reduced myocardial infarct size ($32.7\% \pm 9.1\%$ vs. $48.6\% \pm 12.8\%$, $p < 0.05$) and improved diastolic function as the left ventricle end-diastolic pressure (LVEDP) was lower and maximum rate of decrease of LVP ($-(dp/dt)_{max}$) was higher in SU group (Table). Our findings indicate that NSC_{Ca} channels is involved in development of myocardial dysfunction and arrhythmias, and that targeting SUR1 may provide a new therapeutic approach to ischemic cardiac injury. Table 1. Hemodynamic performance of obese rats after I/R injury

Hemodynamic performance of obese rats after I/R injury		
	Control group (n=22)	SU group (n=22)
LVSP	120.92 ± 13.53	138.12 ± 24.86
LVEDP	6.86 ± 2.42	$2.51 \pm 3.77^*$
(dp/dt) _{max}	2192.39 ± 1461.66	3678.90 ± 1751.96
$-(dp/dt)_{max}$	-1663.62 ± 1097.18	$-2834.77 \pm 1007.23^*$

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Echocardiographic changes in rats with metabolic syndrome observed after long-term cola beverage drinking

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Background and aim: Previously we reported metabolic syndrome-associated alterations in a cola-drinking rat model. Metabolic syndrome has been linked to increased risk of type II diabetes and cardiovascular disease. This work evaluates whether chronic drinking of cola beverages may affect cardiac geometry.

Methods: 48 male Wistar rats drank regular cola beverage (C), light cola beverage (L) or tap water (W) ad libitum (n=16 per group). After 6 months (treatment) 50% of C₆, L₆ and W₆ rats were euthanized. Heart and thoracic and abdominal aorta were excised and harvested for light microscopy (histopathology) at the time of euthanasia. Echocardiograms (ATL 3000 HDI, Bethold, WA, USA) and systolic blood pressure (SBP) (tail cuff plethysmography, electro-sphygmomanometer PE-300, Physiograph MK-IIIS, Narco Bio-Systems, Austin, Texas) were recorded in awake rats at the beginning of the study (baseline) and after treatment (6 months). At the beginning of the study all three experimental groups were indistinguishable from each other according with variables of interest.

Results: C₆ and L₆ groups were respectively different from W₆ group based on: (I). Left ventricular diastolic dimension (LVDD, mm): 7.4 ± 0.3 (+9%, mean diff.= 0.60, CI_{95%}= 1.10 to 0.10, t= 3.19, $p < 0.01$) and 7.3 ± 0.7 (+7%, mean diff.= 0.50, CI_{95%}= 0.99 to 0.09, t= 2.71, $p < 0.05$) vs. 6.8 ± 0.4 ; (II). Left ventricular diastolic volume (LVDV, ml): 0.34 ± 0.04 (+26%, mean diff.= +0.07, CI_{95%}= 0.13 to 0.01, t= 3.32, $p < 0.05$) and 0.33 ± 0.06 (+22%, mean diff.= +0.06, CI_{95%}= 0.12 to 0.01, t= 2.90, $p < 0.05$) vs. 0.27 ± 0.04 ; (III). Systolic volume (SV, ml): 0.31 ± 0.04 (+24%, mean diff.= 0.60, CI_{95%}= 0.12 to 0.01, t= 2.76, $p < 0.05$) and 0.31 ± 0.03 (+24%, mean diff.= 0.60, CI_{95%}= 0.12 to 0.01, t= 2.80, $p < 0.05$) vs. 0.25 ± 0.04 ; (IV). Relative wall thickness (RWTh): 0.37 ± 0.03 (-8%, mean diff.= 0.60, CI_{95%}= 0.003 to 0.077, t= 2.90, $p < 0.05$) and 0.36 ± 0.05 (-10%, mean diff.=

0.60, CI_{95%}= 0.004 to 0.076, t= 2.90, $p < 0.05$) vs. 0.40 ± 0.03 . C₆ was different from W₆ based on cardiac output (ml/min): 148 ± 20 (+29%, mean diff.= +33, CI_{95%}= 66.0 to 0.01, t= 3.06, $p < 0.05$) vs. 115 ± 21 . Treatment had no effect on heart rate (bpm): 470 ± 46 (C₆), 457 ± 44 (L₆), 469 ± 36 (W₆). The necropsy findings were scarce and showed no relation to treatment.

Conclusions: Six months' cola beverage drinking led to increases in diastolic and systolic volumes and left ventricle posterior wall thinning that resulted in larger cardiac output without affecting heart rate. Factors responsible for cardiac remodeling in long-term light cola drinking animals are to be identified. Supported by: CONICET

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Endoglin levels in the kidney are correlated to fibrosis in hypertensive and diabetic rats

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Background and aims: Hypertension is, after diabetes, the second most common cause of chronic renal disease. Renal hypertension puts stress and increases pressure on the kidney. Several authors have suggested the possible link of endoglin, a member of the receptor system of transforming growth factor-beta 1, with different vascular alterations. Endoglin is known to be constitutively expressed in endothelial cells and plays a critical role in both vascular development and endothelial function. Previous studies from our research group showed that endoglin levels are altered in *in vivo* experimental models of diabetes, but there are no studies relating endoglin with the development of renal fibrosis

Materials and methods: We have studied endoglin levels in kidneys from 8 different rat experimental groups: Wistar and Spontaneously Hypertensive Rats (SHR), each divided into 4 experimental groups: sham-operated, diabetic rats (after an injection of 60 mg streptozotocin/kg body weight), uninephrectomized rats (UNX, right kidney removed in order to accelerate the development of disease in the remnant kidney) and UNX diabetic rats. We kept glycaemia levels between 300 and 400 mg/dL in diabetic rats, injecting daily insulin doses. Monthly, both 24 h-urine samples (in metabolic cages) and plasma samples were collected. We assessed renal function by creatinine clearance and determination of proteinuria, albuminuria and blood ureic nitrogen. 8 months after the induction of diabetes, hearts and kidneys were removed, and the expression of endoglin, fibronectin and type I collagen in renal tissue was analyzed (by Western blot and PCR), as well as the presence of left ventricular hypertrophy.

Results: Renal function impairment started at 4th-5th month in UNX rats, either in the presence or absence of diabetes. At the 8th month, renal hypertrophy was observed in UNX groups when compared to Sham rats. We also observed LVH in SHR when compared to Wistar rats. SHR UNX had the largest hearts and, interestingly, diabetic SHR had smaller hearts than their control group (non diabetic SHR). Kidneys of UNX (SHR and Wistar) rats contained larger amounts of fibronectin than kidneys of sham-operated rats. Kidneys from SHR UNX presented the higher expression of type I collagen. Interestingly, endoglin expression was directly correlated with extracellular matrix synthesis, as we observed the highest endoglin expression in SHR UNX kidneys.

Conclusion: These data suggest the existence of a direct correlation between endoglin expression and renal fibrosis in either hypertensive or diabetic rats.

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Hyperbaric oxygen therapy does not worsen the cardiac function and oxidative state of diabetic rats

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Background and aims: Hyperbaric oxygen therapy (HBOT) is frequently used in diabetic patients with ulcerated wounds; however its ability to increase oxidative stress inducing accelerated β -cell death and cardiovascular complications casts doubts.

Materials and methods: Wistar rats (20) weighing 300 g were administered with a single dose of 70 mg/kg streptozotocin to induce diabetes mellitus (DM). The 24-hour blood glucose profile and cardiac function (ultrasound measurements) of diabetic animals and 20 controls (C) were measured three weeks after the induction of diabetes. 10 diabetic (DM HBOT) and 10 controls (C HBOT) underwent one-hour long hyperbaric oxygen treatment (HBOT: 2.5 bar) 12 times (in 16 days). Following HBOT and 8 weeks after streptozotocin treatment, ultrasound and blood glucose measurements were repeated. Blood plasma and lymphocyte samples were collected. Malonyldialdehyde assay (MDA) were performed on the plasma samples and nitrotyrosine immunostaining (NT) was done on lymphocyte smears. For data analysis ANOVA and Tukey's post hoc test were implemented.

Results: Blood glucose concentrations were significantly elevated in both diabetic groups (DM: 23.92 ± 1.91 , DM HBOT: 22.52 ± 1.03 ; $p < 0.0001$ vs. C: 4.84 ± 0.18 , C HBOT: 4.6 ± 0.2). HBOT did not affect blood glucose levels. On the 3rd week of diabetes there were no significant difference between diabetic and control groups in ejection fraction (EF), but stroke volume (SV) (DM: 0.181 ± 0.028 , DM HBOT: 0.183 ± 0.044 ; $p < 0.05$ vs. C: 0.251 ± 0.068 , C HBOT: 0.232 ± 0.054) and end diastolic volume (EDV) (DM: 0.381 ± 0.072 , DM HBOT: 0.404 ± 0.070 ; $p < 0.05$ vs. C: 0.487 ± 0.095 , C HBOT: 0.449 ± 0.099) were significantly lower in diabetic animals. By time the decrement of EF in diabetic rats reached the level of significance. (DM: 32.485 ± 8.206 , DM HBOT: 33.657 ± 10.383 ; $p < 0.0001$ vs. C: 53.339 ± 8.007 , C HBOT: 50.369 ± 9.631) HBOT did not alter the cardiac parameter of control rats. HBOT did not reduced EF and SV in diabetic rats, however it improved EDV (log(EDV) DM: -0.6375 ± 0.0532 vs. DM HBOT: -0.5286 ± 0.09299 , $p < 0.05$). Plasma MDA levels were significantly elevated in diabetic rats compared to both control groups. (DM: 23.5 ± 17.59 ; $p < 0.05$ vs. C: 9.5 ± 4.48 ; $p < 0.01$ vs. C HBOT: 6.1 ± 1) Interestingly HBOT tended to decrease oxidative stress in all animals: there was no significant difference between diabetic HBOT rats and any other group (DM HBOT: 14.8 ± 11.7). Nitrate stress was significantly higher in both DM groups than in the control groups, but NT positivity not altered by HBOT (DM: 38.1 ± 10.1 , DM HBOT: 41.2 ± 7.8 ; $p < 0.05$ vs. C: 20.4 ± 10 , C HBOT: 22 ± 10.5)

Conclusion: HBOT has no negative effect on the cardiac functions of rats having fully developed insulin dependent diabetes mellitus. HBOT neither did increase oxidative-nitrate stress. According to our results HBOT may not increase cardiovascular risk of type1 diabetic patients.

expression of FN. Transfection with miR-146a mimics restored miR-146a level and decreased FN mRNA and protein production, whereas miR-146a antagonists produced a glucose-like effect. Luciferase assay demonstrated binding of miR-146a with FN 3'-UTR. Retinal, cardiac and renal tissues from type 1 and type 2 diabetic animals showed decreased miR-146a and increased FN and p300. Intravitreal injection of miR-146a mimic restored miR-146a expression and prevented retinal FN upregulation. Interestingly, p300 mediated histone acetylation was found to regulate miR-146a expression; whereas another reduced miRNA, miR-200b, which targets p300 was found to control p300 expression.

Conclusion: Data from these experiments demonstrated novel glucose-induced transcriptional circuitry in which histone acetylation and miRNA alterations participate in the regulating extracellular matrix protein production in diabetes. Identifying such molecular mechanisms may lead to potential RNA based treatment for diabetic complications.

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Histone acetylation and miRNA alterations causing increased extracellular matrix protein production in chronic diabetic complications

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Background and aims: In diabetes, increased extracellular matrix protein such as fibronectin (FN) production causes well-known structural abnormalities such as basement membrane thickening, focal scarring and mesangial matrix expansion. At the transcriptional level, epigenetic mechanisms such as histone acetylation and microRNA(miRNA) alteration regulate gene expression and controls several cellular processes. We investigated such alteration in the pathogenesis of increased FN production in diabetes.

Materials and methods: Human umbilical vein endothelial cells and retinal microvascular endothelial cells were exposed to various glucose levels. FN mRNA and protein levels, histone acetylase p300 mRNA and protein levels, histone acetylation, NF- κ B activation, miRNA levels and binding specific miRNA and p300 with FN promoters were assessed. Cells were also subjected to siRNA mediated and chemical blockade of p300 and miRNA mimic or antagonist transfection. Retinal, cardiac and renal tissues from STZ-diabetic rats, db/db mice and respective controls were similarly investigated. Furthermore, retinal tissues were assessed following specific intravitreal miRNA mimic injection.

Results: In both endothelial cells, glucose caused upregulation of p300 along with augmented histone acetylation and binding of p300 and NF- κ B to FN promoter. Such acetylation caused activation of several transcription factors and increased production of FN and other vasoactive factors. In the retina, heart and kidneys of type 1 and type 2 diabetic animals, similar p300 mediated FN upregulation were seen, which were prevented by chemical blockade of p300 or intravitreal injection of p300 siRNA. In addition, glucose caused alterations of several miRNAs in the endothelial cells, including miR-146a, a FN-targeting miRNA which is of importance in inflammation. Glucose caused decrease in miR-146a expression and increased mRNA and protein

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Follistatin-like 1 protects against the induction of cardiomyocyte dysfunction and insulin resistance induced by adipose tissue-derived factors

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Background and aims: Secretory products from epicardial adipose tissue from patients with type 2 diabetes cause cardiomyocyte contractile dysfunction and insulin resistance. These detrimental effects can be ascribed, at least in part, to enhanced release of activin A from epicardial adipose tissue in type 2 diabetes mellitus. Recent proteomic approaches have identified a physiological antagonist of activin A, follistatin-like 1 (Fstl1), as novel adipokine. The present study aimed to investigate whether Fstl1 expression is altered in the intrathoracic adipose tissue depots surrounding the heart of patients with and without type 2 diabetes. Furthermore, we determined whether Fstl1 could protect against the induction of cardiomyocyte dysfunction induced by adipose tissue-derived factors.

Materials and methods: Fstl1 expression was examined in human epicardial, subcutaneous and pericardial adipose tissue biopsies collected from patients with and without type 2 diabetes undergoing open heart surgery (n=4 per group) by Western blotting. Primary rat cardiomyocytes were exposed to conditioned media generated from abdominal adipose tissue from high-fat diet-fed guinea pigs to induce cardiomyocyte dysfunction. To assess whether Fstl1 protects against these cardiopressive effects, insulin-stimulated Akt-phosphorylation and contractile function were determined in cardiomyocytes exposed to conditioned media in the presence and absence of 100 ng/ml recombinant Fstl1.

Results: Fstl1 was highly and selectively expressed in epicardial adipose tissue relative to subcutaneous and pericardial adipose tissue. Furthermore, expression of Fstl1 was reduced by 50% in epicardial adipose tissue from type 2 diabetic patients compared to non-diabetic subjects (P<0.05). In cardiomyocytes, Fstl1 enhanced insulin-induced phosphorylation of Akt by 30% (P<0.01), but had no effect on sarcomere shortening and cytosolic Ca²⁺-transients. Incubation of cardiomyocytes with conditioned media lowered insulin-induced Akt-phosphorylation by 30% (P<0.005), and induced contractile dysfunction as illustrated by reductions of 72% and 38% in sarcomere shortening and cytosolic Ca²⁺-influx after electrical stimulation, respectively (both P<0.001). Fstl1 restored the reductions in sarcomere shortening and cytosolic Ca²⁺-transients to 75% and 90%, respectively, of the response induced by control medium (both P<0.001). Finally, the abrogation of insulin-mediated Akt-phosphorylation by conditioned media was completely prevented when Fstl1 was present (P<0.001).

Conclusion: Collectively, these findings indicate that the novel adipokine Fstl1 has a cardioprotective function, and that a loss of Fstl1 in epicardial adipose tissue from type 2 diabetic patients could contribute to the development of cardiac dysfunction in type 2 diabetes mellitus.

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Gender-specific differences in the effects of acute hyperglycaemia and hyperinsulinaemia on myocardial lipid content and function

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Background and aims: Women with diabetes display an increased vulnerability for heart failure compared to men. Furthermore, increased myocardial lipid content (cardiac steatosis) has been recently found to play a causal role in development of diabetic cardiomyopathy and is more pronounced in women with overt hyperglycemia compared to their male counterparts. Hence, the aim of this study was to investigate the impact of acute hyperglycemia and associated hyperinsulinemia on myocardial lipid content and left ventricular function in healthy men and women.

Materials and methods: Hyperglycemic clamps (6 hours) were performed in 18 healthy subjects (8 women, 10 men). Left ventricular dynamic parameters

and myocardial lipid content in cardiac septum were assessed by ¹H magnetic resonance imaging and breath movement navigated and ECG triggered localized ¹H single voxel MR spectroscopy, respectively; at baseline as well as during the sixth hour of hyperglycemia.

Results: Hyperglycemia (mean glucose: women: 201.4±5.3, men: 202.5±13.8 mg/dl; ns) and hyperinsulinemia (mean insulin: women: 123.5±34.9, men: 99.3±74.9 μU/ml; ns) were associated with a similar (ANOVA: ns) suppression of circulating free fatty acids (women: -93.4%, p=0.0004; men: -85.2%, p=0.002,) and a comparable (ANOVA: ns) increase in myocardial lipid content (women: +29.1%, p=0.018; men: +39%, p=0.01,) in both, men and women, in whom also age (women: 29.0±7.2, men: 27.6±3.4 years; p=ns) and Body-Mass-Index (women: 21.4±2.3, men: 23.1±2.4 kg/m²; p=ns) were comparable. However, only in women, ejection fraction significantly increased (+5.6%, p=0.0008) and end-systolic volume significantly decreased during hyperglycemia (-14.1%, p=0.0015). The increase of ejection fraction was found to be positively correlated with the rise of insulin concentrations during the clamp in the whole study group (R=0.7, p=0.002), while no impact of the infused volume on myocardial function parameters was observed.

Conclusion: In conclusion, acute hyperglycemia and hyperinsulinemia induce a comparable short term increase in myocardial lipid content in both, healthy women and men, indicating that these metabolic alterations might be directly responsible for myocardial steatosis in type 2 diabetes. However, short term changes in myocardial function (that might be related to hyperinsulinemia) were observed only in women.

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Effect of diabetes and hyperglycaemia on the left ventricle - does it differ with ethnicity?

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Background and aims: Existence of a distinct diabetes related cardiomyopathy, and specifically, an increase in left ventricular (LV) mass as a consequence of hyperglycaemia, is controversial. In addition, the contributions of hypertension and obesity to an increase in LV mass in association with hyperglycaemia have not wholly been accounted for. Understanding how the clusters of these risk factors associated with diabetes interact on ventricular structure can be assisted by studying ethnic minority groups where risk factor clustering differs markedly. We therefore explored the relationship between hyperglycaemia with LV mass in three ethnic groups (European (E), Indo Asian (IA) and African Caribbean (Afc) using both 2D and real time 3D echocardiography.

Materials and methods: A population based sample of 1,277 men and women (608 E, 462 IA and 207 Afc) aged 58-86 years underwent 2D echocardiography (iE33, Philips). LV mass was calculated from the parasternal long axis view using diastolic wall thickness and cavity diameter measures. In addition, 726 of these participants (389 E, 239 IA and 98 Afc) underwent real time 3D echocardiography. 3D full volume sets were acquired from the apical 4 chamber view with a matrix array transducer and LV mass was measured directly offline (Philips Qlab 7.0). Both 2D and 3D LV mass measures were indexed to height^{2.7} (g/m^{2.7}). Height, weight and blood pressure were measured and an OGTT performed.

Results: Using conventional 2D measures, LV mass increased with advancing glucose intolerance in all ethnic groups. People with diabetes had greater LV mass than normoglycaemic individuals by +5.1g/m^{2.7} in E, +5.2g/m^{2.7} in IA and +5.2g/m^{2.7} in Afc. Within each ethnic group LV mass/height^{2.7} was strongly associated with age, BMI, systolic blood pressure, fasting blood glucose and HbA_{1c} (table). However the association of glucose and HbA_{1c} did not remain significant after additional adjustment for BMI (table). BMI also accounted for the ethnic differences in LV mass, for example, BMI in normoglycaemic individuals was largest in Afc (28.8±0.6kg/m²), followed by E (26.8±0.2kg/m²) and was smallest in IA (25.8±0.3kg/m²). 3D echocardiography in people without known diabetes also showed a significant association between LV mass/height^{2.7} and glucose (β=0.7±0.3 p=0.025) that was abolished following adjustment for BMI (β=0.12±0.3 p=0.69).

Conclusions: Diabetes and hyperglycaemia are associated with an increase in LV mass. The association between LV mass and hyperglycaemia was similar by ethnic group. Within ethnic groups, BMI accounted for the association between hyperglycaemia and LV mass. BMI also accounted for the absolute ethnic differences in LV mass by glucose tolerance category.

2D LV mass/height ^{2.7}	European (n=608)			Indo Asian (n=462)			African Caribbea n (207)		
Glucose status	Mean±SD	p-value		Mean±SD	p-value		Mean±SD	p-value	
Normal	43.1±0.6			40.7±0.7			45.5±0.9		
Pre-diabetes	44.9±0.7	0.14		42.6±0.8	<0.0001		47.3±1.0	0.374	
Newly diagnosed Diabetes	46.6±1.2	0.01		44.2±1.2	0.1		49.0±1.4	0.333	
Treated Diabetes	48.2±0.9	<0.0001		45.9±0.8	<0.0001		50.7±1.0	0.559	
Regression Analysis	β	SE	p-value	β	SE	p-value	β	SE	p-value
BMI (Kg/m ²)	1.4	0.05	<0.0001	0.9	0.11	<0.0001	1.02	0.14	<0.0001
SBP (mmHg)	0.12	0.03	<0.0001	0.16	0.03	<0.0001	0.23	0.05	<0.0001
Fasting Glucose (mmol/l)	11.4	2.6	<0.0001	4.23	2	0.03	7.8	3.8	0.04
Fasting Glucose, BMI	2.02	2.34	0.39	2.03	1.9	0.28	4.5	3.5	0.21
HbA1C (%)	20.6	4.3	<0.0001	9.7	3.2	0.003	10.9	5.6	0.054
HbA1C,BMI	7.5	3.9	0.05	6.2	3.1	0.05	6.3	5.3	0.24

All LV mass/height^{2.7} values are age and sex adjusted. all regressions are age adjusted

All LV mass/height^{2.7} values are age and sex adjusted, all regressions are age adjusted

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Evaluation of vascular inflammation using FDG-PET in primary coronary heart disease prevention

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Background and aims: To evaluate vascular inflammation by ¹⁸F-fluorodeoxyglucose (FDG) positron emission tomography (PET) CT, which reflects vulnerable atherosclerotic plaque in primary coronary heart disease prevention.

Materials and methods: We measured high-sensitivity C-reactive protein (hsCRP) and traditional cardiovascular risk factors in 142 healthy subjects. To assess the vascular influence of hsCRP on each Framingham risk score (FRS) category, we compared carotid intima-media thickness (CIMT), brachial-ankle pulse wave velocity (baPWV), and vascular inflammation, which was represented as the target-to-background ratio (TBR) measured using FDG-PET/CT.

Results: In both intermediate (10%–20%) and low (<10%) FRS categories, mean TBR values in subjects with higher hsCRP levels (≥2 mg/L) were significantly increased compared to those with lower hsCRP levels (<2 mg/L) (P = 0.001, P < 0.001, respectively). However, baPWV and CIMT values did not significantly differ according to hsCRP levels in the same FRS categories. Mean TBR levels positively correlated with FRS, body mass index (BMI), waist circumference, LDL-cholesterol, triglyceride, creatinine, glucose, and hsCRP, whereas negatively correlated with HDL-cholesterol. Multiple stepwise regression analyses showed that hsCRP, LDL-cholesterol, BMI, and insulin resistance were independently associated with mean TBR values (R² = 0.414).

Conclusion: In both intermediate and low FRS risk groups, vascular inflammation measured using FDG-PET/CT was increased in individuals with higher hsCRP levels compared to those with lower hsCRP.

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The influence of metabolic disorders in children with diabetes mellitus type 1 on changes of QT interval

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Diabetes Mellitus disturbs cellular metabolism of all organs and tissues, including myocardium.

Research: to evaluate values of intervals QT and QTc in children with Diabetes Mellitus type 1 (DM1) and to reveal interrelation of the given changes with the duration of disease, age, sex, indices of metabolic control.

Materials and methods: QT and QTc were evaluated on ECG in 178 children with DM1 (middle age 13.44±0.29 years, duration of disease 5.72±0.28 years) and in 60 sex- and age-matched healthy children. Level of HbA_{1c} in children with DM1 made up 9.82±0.18% (N<6.2%; P<0.001).

Results: Increases of values of intervals QT and QTc are characteristic to children with DM1 in comparison with control group (364.56±2.65 ms and 421.39±3.1 ms, 352.97±15.1 ms and 392.73±13.0 ms respectively, P<0.0015). It is established that values of QTc in girls with DM1 are higher than in boys (428.07±4.51 ms versus 413.6±4.08 ms, P<0.0025). 17.71% of girls and 13.41% of boys with DM1 have autoimmune thyroiditis (the percent of children with values of QTc>440 ms is high in this group). Levels of thyroid-stimulating hormone (TSH) and thyroperoxidase antibodies (Anti-TPO) are different before and after manifestation of Diabetes Mellitus type 1 (4.21±0.44 μIU/ml and 83.13±13.45 IU/ml, 5.52±1.06 μIU/ml and 251.62±32.66 IU/ml respectively, P<0.0002) At the same time values of HbA_{1c} are higher in boys than in girls (10.4±0.3% and 9.35±0.2% respectively, P<0.002). The difference in pulse rate between girls and boys is insignificant for the group studied. Connection of QTc with age (r=0.338, P<0.00001) and level of HbA_{1c} (r=0.37, P<0.0001) was established. Feedback connection of QT with pulse rate was revealed (r=-0.48, P<0.005). There were no reliable correlations of QTc with Body Mass Index, level of cholesterol, duration of the disease and values of systolic and diastolic blood pressure.

Conclusions: The increase of QT and QTc is noticed in children with DM1. Age, level of glycemic and pulse rate are the factors defining values of QTc. In girls QTc has higher values more often than in boys. Intervals QTc>440 ms are found more often in children with DM1 and autoimmune thyroiditis.

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Coronary response to an increased oxygen demand in asymptomatic type 2 diabetic patients: a new non-invasive approach

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Rationale and aims: The aim of the present study was to assess, using for the first time a non invasive method, the coronary response to physiological increase in myocardial demand, and its relationship with silent myocardial ischemia (SMI) and silent coronary stenoses (CS) in type 2 diabetic patients (T2D).

Materials and method: A total of 118 asymptomatic T2D for 15.0±7.4 years (72 males, 61.1±8.2 years, BMI 30.6±5.4 kg/m²) without a cardiac history and fulfilling the criteria of the French guidelines for SMI screening were prospectively screened for SMI, defined as an abnormal stress myocardial scintigraphy and/or stress echocardiography. A coronary angiography was performed in those with SMI. The distal inter-ventricular anterior coronary velocity (CV) was measured by trans-thoracic echo-doppler (3-7MHz) before and after cold pressure test (CPT). CPT was also performed in 30 overweighted or obese non diabetic subject (OS) and 25 control subjects < 40 years.

Results: Thirty-five of T2D had SMI, and 15 of them CS. CV during CPT could be measured in 56% T2D, 37% OS and 64% controls. Changes after CPT/baseline in DP (rate-pressure product) (43±034, 36±27 and 32±19% increase) and in CV (30±26, 21±34 and 17±10% increase) did not significantly differ in the 3 groups. CV changes correlated with DP changes in control (r=0.58, p<0.05), OS (r=0.75, p<0.01), T2D without CS (r=0.56, p<0.0001) but not in those with CS. In T2D, SMI was associated with higher increase in CV after CPT (SMI- 21±22, SMI+CS- 36±28 and SMI+CS+ 39±26% increase, p=0.03) despite similar DP increase, with male gender, BMI, diabetic nephropathy, triglycerides and haptoglobine (p=0.02 to 0.05). In multivariate analysis including these factors, SMI was only associated with CV changes (per 10% increase OR: 1.3 [1.1–1.5], p<0.05).

Conclusion: 1) Non invasive study of the coronary reactivity is feasible by trans-thoracic echography-doppler with a lower feasibility in OS, 2) the coronary response to an increase in myocardial oxygen demand is impaired in T2D patients with silent CS, 3) T2D with SMI in particular those with CS have higher increase in coronary velocity after CPT, probably due to the lack of coronary dilation related to endothelial dysfunction.

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Co-morbidity burden and incident hypoglycaemia in patients with type 2 diabetes and heart failure: insight from DiaRegis

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Background and aims: Data on co-morbidity burden and hypoglycaemia in particular in patients with type 2 diabetes and heart failure (HF) are scarce.

Materials and methods: DiaRegis is a prospective registry in type-2 diabetic patients insufficiently controlled on oral mono- or dual antidiabetic therapy. Patients with or without anamnestic HF were compared.

Results: For 3,746 patients, 9.9% of which had HF, data were available. Median age was 65.9 years, 46.8% were female. HF patients were older (73.1 vs. 64.7 years; $p < 0.0001$) and frequently had co-morbid disease such as hypertension (95.4 vs. 83.1%; $p < 0.0001$), coronary heart disease (52.9 vs. 13.8%; $p < 0.0001$), prior stroke/TIA (9.5 vs. 4.1%; $p < 0.0001$), peripheral arterial disease (15.6 vs. 4.9%; $p < 0.0001$) and autonomous neuropathy (7.2 vs. 2.9%; $p < 0.0001$). Prior to enrolment into the registry, they received less metformin (Met) and more sulfonylureas (SU) in monotherapy compared to patients without HF, both drugs are known to interfere with the incidence of HF. After enrolment insulin treatment was initiated in 21.4% patients with HF versus 16.6% patients without HF ($p < 0.05$). Incident stroke/TIA, angina pectoris, percutaneous coronary intervention, and peripheral angioplasty were significantly increased in patients with HF after a 6 months follow-up. Furthermore, hypoglycaemic events were 50% more frequent in patients with HF in the 12 months preceding enrolment and at the 6 months follow-up.

Conclusion: Patients with type 2 diabetes and HF show a substantial co-morbidity and seem to be more prone to develop hypoglycaemia.

	Heart failure (n=370)	No heart failure (n=3386)	p-value
Metformin enrolment / after therapy change at baseline (%)	76.8 / 74.3	85.0 / 85.8	<0.0001 / <0.0001
Sulfonylurea prior enrolment / after therapy change at baseline (%)	32.7 / 23.0	28.3 / 26.6	0.07 / 0.13
DPP4-inhibitors prior enrolment / after therapy change at baseline (%)	7.3 / 37.6	4.7 / 39.2	<0.05 / 0.55
Hypoglycaemia (within 12 months prior enrolment) (%)	17.8	10.0	<0.0001
6-months FU			
Stroke / TIA (%)	1.4	0.3	<0.01
Stable angina (%)	3.7	1.0	<0.0001
Unstable angina (%)	2.3	0.2	<0.0001
PCI (%)	1.1	0.2	<0.01
Peripheral angioplasty (%)	2.0	0.4	<0.0001
Hypoglycaemia (%)	12.1	8.1	<0.05

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Lower incidence of myocardial infarction in type 2 diabetic patients with polyneuropathy who were treated with an aldose reductase inhibitor (epalrestat): a retrospective study

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Background and aims: Epalrestat, an aldose reductase inhibitor, is clinically available only in Japan, and has been being used since 1992 for treatment of diabetic polyneuropathy (DP). Epalrestat inhibits enhanced aldose reductase activity, reduces accumulation of sorbitol in tissues, and suppresses progres-

sion of DP in diabetic animals and human. Furthermore, epalrestat has anti-oxidant activity, and it has been indicated that epalrestat has a protective effect on arteriosclerosis and ischemia/reperfusion injury. These imply that epalrestat may have a protective effect against myocardial infarction (MI) in diabetic patients. Therefore, we retrospectively studied incidence of MI in patients with type 2 diabetes with DP, who were treated or untreated with epalrestat for more than 5 years, and compared the incidence between the two groups.

Materials and methods: Subjects were patients with type 2 diabetes and DP, which was defined by the practical and simple diagnostic criteria proposed by the Diabetic Neuropathy Study Group in Japan. Diabetic patients were diagnosed as having DP, when they had two of three items: (1) sensory symptoms considered to be due to DP, (2) bilaterally decreased or absent ankle reflex and (3) decreased vibratory sensation in bilateral medial malleoli, and when other neuropathy than DP could be excluded. There were no difference in age, sex, duration of diabetes, and HbA1c between the patients treated with epalrestat (150 mg/day) (N = 111) and without epalrestat (non-epalrestat) (N = 64).

Results: The incidence of MI was 3.5 (per 1000 x person x year) in the epalrestat group treated with epalrestat for more than 5 years, whereas it was 12.8 (per 1000 x person x year) in the non-epalrestat group. On the other hand, there was no difference in the incidence of cerebrovascular diseases in the two groups (8.9 vs. 9.3). The Kaplan-Meier analysis indicates that there was a significant difference in the incidence of MI (Logrank test, $p = 0.019$), but not cerebrovascular diseases (Logrank test, $p = 0.919$), between the two groups. Prevalence of DP, retinopathy, and nephropathy was 100% vs. 100% (NS), 57.7% vs. 59.4% (NS), and 49.5% vs. 82.8% ($P < 0.01$) in the epalrestat and the non-epalrestat group, respectively. There were significant differences in proportion of patients taking oral hypoglycemic agents (98.2% vs. 56.3%, $P < 0.01$) and anti-hypertensive drugs (60.4% vs. 31.3%, $P < 0.01$) between the epalrestat and the non-epalrestat group, whereas no differences in that of patients receiving insulin (55.0% vs. 56.3%, NS) and taking cholesterol-lowering drugs (45.9% vs. 31.3%, NS) in the two groups. A multiple regression analysis is in progress to reveal an independent association of the use of epalrestat with low incidence of MI.

Conclusion: The results imply that epalrestat may have protective effect against MI in patients with type 2 diabetes and PN. It will be worthy to evaluate a beneficial effect of epalrestat on prevention of MI in diabetic patients by a randomised prospective clinical study.

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Metformin, Vit B₁₂ deficiency and silent myocardial ischaemia in type 2 diabetic patients

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Objective: To investigate the relationship between metformine-associated vitamin B12 (Vit B12) deficiency and hematological and cardiovascular morbidity in type 2 diabetes.

Research design and methods: 795 patients with type 2 diabetes (57.6 % male, 62.3 % metformin-treated, mean HbA1c 8.3 ± 1.5 %) with > 1 cardiovascular risk factor were evaluated in this retrospective cross-sectional study. Their main characteristics are shown in the table. They all underwent silent myocardial ischemia (SMI) screening using dipyridamole combined with exercise myocardial perfusion imaging (MPI). SMI was defined as positive MPI (mean activity < 70% of the maximal myocardium activity in ≥ 3 of 20 segments) or positive exercise electrocardiogram (ECG) (horizontal or descending ST segment depression > 1 mm). Biological parameters were evaluated in fasting conditions on the same day as SMI screening. Glomerular filtration rate (GFR) was estimated by the Cockcroft and Gault formula. Vit B12 deficiency and hyperhomocysteinemia were defined as values < 150 pmol/l and > 15 μ mol/l, respectively. Adjustments were made using multivariate logistic regressions.

Results: Crude prevalences of Vit B12 deficiency were 12.5 % in metformin-treated patients and 6.1 in non-metformin-treated patients ($p < 0.01$). After adjustments for tobacco use, gender, BMI, age, GFR, insulin use and presence of hypertension, metformin use was associated with a significant risk of Vit B12 deficiency (OR 3.22; 95% CI = 1.44- 7.19). In metformin-treated population, Vit B12 deficient were older (66.7 ± 7.7 vs 60.1 ± 8.8 , $p < 0.05$), with a longer duration of diabetes (16.7 ± 11.1 vs 12.9 ± 9.1 , $p < 0.001$) and a higher AST/ALT ratio (1.14 ± 0.47 vs 0.94 ± 0.35 , $p < 0.001$). Metformin-associated Vit B12

deficiency was not associated with a higher prevalence of hyperhomocysteinaemia (crude values 52.6 vs 34.6 %, OR 1.81 [95% CI = 0.62– 5.26] adjusted for age, nephropathy, AST/ALT ratio, erythrocytes folates) or with a higher risk of SMI (crude values 13.3 vs 22.6 %, OR 0.41 [95% CI = 0.14– 1.16] adjusted for age, gender, BMI, nephropathy, LDL-cholesterol, tobacco use, presence of hypertension, hs-CRP). There was no association between Vit B12 deficiency and anemia (24% vs 20% $p = 0.51$) or macrocytosis (4.7% vs 2.5% $p = 0.35$).

Conclusion: This cross-sectional study confirms the association between metformin and Vit B12 deficiency. In our series, metformine-associated Vit B12 deficiency is associated neither with an increased prevalence of silent myocardial infarction nor with hematological abnormalities.

two subgroups. Similarly, comparing patients in subgroup 2 to the ones in subgroup 3 there were no significant differences found in the echocardiography parameters measured neither at the beginning of the study nor after 3 years of follow up. Patients in subgroup 2 had no significant differences in echocardiography parameters found during the 3 years of observation.

Conclusion: The longer existence of glucose metabolism disorders in patients after kidney transplantation the worse are the outcomes of some of the diastolic myocardial function parameters (E/A wave ratio, IVRT) and paradoxically the thickness of left ventricular posterior wall and septum improves with time. Further, larger studies should provide verification of this finding and clues on its nature.

Comparison of non-metformin-treated and metformin-treated patients

	All patients (N = 798)	Non-metformin-treated (N = 301)	Metformin-treated (N = 497)	p
Age, mean (SD), years	62.6 (9.6)	64.7 (9.9)	61.5 (9.2)	<0.001
Diabetes duration, mean (SD), years	14.3 (9.5)	15.4 (9.9)	13.6 (9.1)	<0.05
BMI, mean (SD), kg/m ²	30.4 (5.8)	29.1 (5.7)	31.2 (5.7)	<0.001
Glomerular filtration rate, mean (SD), ml/min	102 (46)	87 (42)	112 (45)	<0.001
Co-treatment with insulin (%)	48.9	70.4	36.0	<0.000001
Co-treatment with sulfonylurea (%)	43.5	22.3	56.5	<0.000001
Proteinuria (%)	13.7	19.9	9.8	<0.001
Anemia (%)	21.8	25.7	19.5	0.06
Adjusted Vit B12, mean (SEM), pmol/l		386 (15.0)	323 (13.0)	<0.001
Homocysteine, mean (SD), μ mol/l	14.5 (5.3)	14.9 (5.3)	14.3 (5.2)	NS
Silent myocardial ischemia (%)	20.1	16.6	21.8	0.08

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Glucose metabolism disorders and echocardiography parameters in patients after kidney transplantation during 3-years of follow up

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Background and aims: Glucose metabolism disorders are one of the diseases that accelerates arteriosclerosis. The study aimed to assess if glucose metabolism disorders in patients after kidney transplantation are associated with worsening of heart function in echocardiography examination.

Materials and methods: This was a 3-year prospective, observational study in the group of 83 patients (53 men and 31 women; aged 45.6 ± 12.1 years) in mean 5.3 ± 2.3 years after kidney transplantation with chronic graft dysfunction (GFR 49.3 ± 19 ml/min/1.73m²) receiving the same immunosuppressive therapy. The echocardiography parameters, kidney function (GFR) and diabetic status (OGTT, HbA1c, postprandial glycaemia) were monitored once a year and compared between the groups of patients with and without glucose metabolism disorders with adjustment for age, sex, BMI, smoking, hypertension, and lipid status (analysis of covariance).

Results: At the beginning of the study there were 15 patients with glucose metabolism disorders of mean duration 5.0 ± 3.9 years (subgroup 1): diabetes type 2 ($n=12$), impaired fasting glucose (IFG, $n=2$) and impaired glucose tolerance (IGT, $n=1$). After 3 years of follow up there were 14 new cases of glucose metabolism disorders revealed (subgroup 2): post-transplant diabetes mellitus ($n=6$), IFG ($n=5$) and IGT ($n=3$). There were 54 patients without glucose metabolism disorders (subgroup 3). Patients in subgroup 1 comparing to the ones in subgroup 2 had significantly worse E/A wave ratio after 3 years of follow up (0.7 ± 0.2 vs 1.18 ± 0.4 , $P=0.010$) while at the beginning of the study no difference in diastolic heart function parameters were found. At the beginning of the study there was posterior wall diastolic diameter (PWDd) significantly thicker in subgroup 1 comparing to subgroup 2 (13.9 ± 0.9 mm vs 12.3 ± 0.4 mm, $P=0.047$) but the difference paradoxically became not significant after 3 years of follow up. Comparing the echocardiography measurements among patients in subgroup 1 there was difference in diastolic heart function found where IVRT became significantly longer after 3 years of follow up comparing to the beginning of the study (110.96 ± 17.5 msec vs 101.7 ± 15.1 msec, $P=0.047$). Comparing patients in subgroup 1 to the ones in subgroup 3 there was PWDd (13.9 ± 0.9 mm vs 12.6 ± 0.5 mm, $P=0.029$) and intraventricular septum diastolic diameter (14.2 ± 0.8 mm vs 12.6 ± 0.4 mm, $p=0.020$) significantly thicker at the beginning of observation but after 3 years of follow up there was no significant differences in relation to left ventricular wall or septum thickness between the

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Blood glucose fluctuation-induced apoptosis in myocardium and aorta through oxidative stress and chronic inflammation

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Background and aims: Patients with diabetes are at increased risk of cardiovascular disease. Recent studies have shown that the development of cardiovascular complications in diabetes is not only associated with persistent high blood glucose level, but also closely associated with the fluctuations of blood glucose. Most previous studies of the effect of blood glucose fluctuation on vascular endothelial cells are done in vitro, few studies have reported the effects of intermittent hyperglycemia on the apoptosis of myocardium and aorta in vivo. Therefore, we established a model in vivo of blood glucose fluctuation by intermittent high glucose infusion in Wistar rats. Co-infusion with N-acetyl cysteine (NAC, an antioxidant) was designed to investigate the effects of NAC on apoptosis of myocardium and aorta, and to explore its potential mechanism of blood glucose fluctuation on these tissues in vivo.

Materials and methods: Cannulated rats (n=6-8/group) were infused for 48h intravenously with (1) saline(SAL), or (2) 50% glucose continuously, maintaining persistent high blood glucose level at 20 ± 0.5 mmol(PHG), or (3) 50% glucose intermittently, maintaining blood glucose level at 5.5 ± 0.5 mmol for 1h, then at 20 ± 0.5 mmol for 1h, changing the level of blood glucose alternatively(IBG), or (4) IBG plus NAC, $0.35 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (IBG+NAC). Oxidative stress measured as the concentration of malondialdehyde (MDA), nitric oxide (NO) and glutathione peroxidase (GSH-PX) activity in serum and cardiac and aortic tissues was analyzed using colorimetric method. We also evaluated levels of nuclear factor- κ B (NF- κ B), and intercellular adhesion molecule-1 (ICAM-1) with immunohistochemistry, the expression of IL-6 mRNA and TNF- α mRNA with RT-PCR, the levels of IKK β , Bax and Bcl-2 with western blot, and apoptosis with TUNEL in myocardium and aorta.

Results: The results showed that both IBG and PHG groups had higher levels of oxidative, inflammatory markers and apoptotic proteins in myocardium and aorta than control group ($P < 0.05$). In myocardium and aorta, the levels of oxidative markers (MDA, NO) were significantly higher, while GSH-PX activity was lower in IBG group than those in PHG and control groups ($P < 0.05$). Compared with PHG and control groups, the levels of inflammatory markers (NF- κ B, IKK β , ICAM-1, TNF- α mRNA and IL-6 mRNA) and pro-apoptotic protein (Bax) in IBG group were increased remarkably ($P < 0.05$), while the anti-apoptotic protein (Bcl-2) was lower ($P < 0.05$). The number of apoptotic cells of myocardium and aorta was higher in IBG group ($P < 0.05$). Co-infusion with NAC prevented the elevation oxidative and inflammatory markers in IBG group. NAC also inhibited the elevation of Bax and the decrease in Bcl-2, meanwhile, abolished apoptosis of myocardial and aortic cells in IBG group.

Conclusion: Blood glucose fluctuations induced more severe oxidative stress, chronic inflammation and apoptosis in myocardium and aorta than persistent high blood glucose. NAC, as an antioxidant, prevented apoptosis of myocardium and aorta induced by blood glucose fluctuation in vivo. This effect of NAC might due to its antioxidative and anti-inflammatory properties.

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1245

Postprandial phase fluctuations can trigger the coagulation cascade

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Background and aims: Cardiovascular Diseases (CVD) are the most common causes of mortality and morbidity among patients with type 2 diabetes. Poorly controlled postprandial hyperglycemia contributes to the development of atherosclerosis. Fluctuations of the postprandial glucose levels bring changes in the coagulation system and propensity to thrombosis. Our aim was to determine the change of plasma coagulation parameters like D-Dimer, β -Thromboglobulin (β -TG), P-Selectin, Plasminogen activator inhibitor-1 (PAI-1), Prothrombin fragments 1-2 (PTF 1-2) in comparison to the fasting

levels in 15 healthy controls and type 2 diabetic patients under treatment of various agents (metformin, insulin secretagog agents and insulin).

Materials and methods: Blood samples were withdrawn after 12 h of fasting (min 0) and following breakfast composed of foods proper for each person, at 60th, 90th and 120th minutes. Fasting and 60th, 90th, and 120th minute measurements of glucose, insulin, triglyceride, D-Dimer, β -TG, P-Selectin, PAI-1, PTF 1-2 had been performed. HA1C and fructosamine were measured also.

Results: Some coagulation parameters tend to be changed at the postprandial phase in diabetics as well as in healthy controls. At the postprandial phase, PAI-1 increased and β -TG decreased significantly in both healthy controls and in all groups of diabetics. The fasting levels of fibrinogen, D-Dimer and P-Selectin were high in diabetics in comparison to healthy controls. An increase in the levels of P-Selectin, PAI-1 and PTF 1-2 at the postprandial phase was observed in healthy persons. Patients receiving insulin secretagog therapy showed an increase in the postprandial levels of PAI-1 like healthy controls. Patients receiving metformin showed an increase in the postprandial levels of PAI-1 and PTF 1-2. Postprandial phase changes in patients receiving metformin were similar to healthy controls. Poorly controlled, older patients with longer diabetes duration had been receiving insulin and these mentioned patients' levels of fibrinogen, D-Dimer and P-Selectin were high in the fasting state and showed an increase in PAI-1 at the postprandial phase. Postprandial levels of PTF 1-2 and D-Dimer were high in insulin treated patients. Levels of fibrinogen and D-Dimer were higher in patients with retinopathy. HA1C and fructosamine were correlated with the coagulation parameters like P-selectin, PAI-1 and PTF 1-2 levels. Correlations showed us that not only postprandial hyperglycemia but also accompanying diabetes, obesity, dyslipidemia and hypertension can aggravate this coagulation tendency at the postprandial phase.

Conclusion: Postprandial phase changes can trigger postprandial coagulation cascade in diabetics as well as healthy persons.

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Analogue versus human insulin regimen improves postmeal glucose and cardiac function in type 2 diabetes patients with intensive conventional insulin therapy

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Background and aims: Fast acting analogue insulins lower postprandial (pp) glucose levels more effectively also in type 2 diabetes (T2D) compared to regular human insulin. However, it is not clear whether this therapy is also able to improve myocardial dysfunction in T2D. This prospective, randomized, open long-term study tested the hypothesis, that intensive conventional insulin therapy (ICT) with analogue insulins (AI) vs. human insulins (HI) improves pp glucose control and cardiac function.

Materials and methods: For 36 months, 109 T2D patients were seen every three months to adapt ICT treatment for the targets fasting glucose ≤ 110 mg/dl and pp glucose ≤ 150 mg/dl in the two groups: AI (insulin detemir and insulin aspart, n=61) and HI (NPH-insulin and regular human insulin, n=48). Diastolic myocardial function (E') was assessed by pulsed tissue Doppler before and two hours after a standardized meal, a pure carbohydrate continental type of breakfast (48g carbohydrates). Intact proinsulin was determined in representative subgroups of AI (n=36) and HI (n=33). Both groups were comparable with regard to demographics, cardiac function and metabolic control at baseline. Data were analyzed by multiple imputation technique.

Results: After 36 months, pp serum glucose was reduced by 20 ± 64 mg/dl ($p < 0.03$) in AI but increased by 4 ± 56 mg/dl (n.s.) in HI (AI vs. HI $p < 0.05$). Fasting serum glucose was reduced by 12 ± 53 mg/dl in AI ($p < 0.096$) and by 19 ± 44 mg/dl in HI ($p < 0.01$). HbA1c decreased in AI from 7.3 ± 1.7 to $6.7 \pm 0.8\%$, $p < 0.02$) and in HI (7.7 ± 1.8 to $6.5 \pm 0.8\%$, $p < 0.001$). In AI, E' increased from 7.8 ± 1.4 to 8.3 ± 1.7 cm/s ($p < 0.02$) in the fasting state and pp from 7.6 ± 1.6 to 8.3 ± 1.7 cm/s ($p < 0.002$). In HI, there was no change of E' fasting (8.2 ± 1.7 to 8.1 ± 1.8 cm/s) or pp. Intact proinsulin was significantly more reduced in AI compared to the increase in HI ($-0.7 [-2.3 - 0.8]$ vs. $0.3 [-0.9 - 2.7]$ pmol/l, $p < 0.049$).

Conclusion: After 36 months, ICT with AI significantly improved pp glucose control associated with an improved insulin resistance and cardiac function compared to ICT with HI. Given the prognostic relevance of diastolic myocardial dysfunction in T2D, this association should be taken into consideration when selecting an insulin regimen for people with T2D.

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Hypoglycaemia and cardiovascular disease: results from the Catalan National Health Service registry on insulin pump therapyI. Conget¹, J. López², C. Castell³, M. Giménez¹¹Diabetes Unit, Endocrinology Department, Hospital Clínic i Universitari/IDIBAPS, Barcelona, ²Endocrinology and Nutrition Department, University Hospital of Leon, ³Health Department, Catalan Health Service, Generalitat de Catalunya, Barcelona, Spain.

Background and aims: The concept that repeated hypoglycaemia could be a potential aggravating factor for atherosclerotic processes in diabetes (type 1 and type 2, T1D) has recently been suggested. Recurrent severe hypoglycaemic episodes and hypoglycaemia unawareness are usually considered indications for continuous subcutaneous insulin infusion (CSII) therapy. The aim of our study was to evaluate the prevalence of cardiovascular disease (CVD) in patients with and without history of repeated hypoglycaemia at the time of starting CSII therapy.

Materials and methods: The database from the Catalan National Health Service registry on CSII therapy (1990–2010) was analysed. It included 1550 T1D patients at entry and prior starting CSII (Aged 34 ± 12 years; 69% women, duration of diabetes 16 ± 9 years; HbA_{1c} 8.2 ± 1.3 %; body mass index (BMI) 24 ± 5 kg/m²). The percentage of patients in which the main indication for CSII included repeated hypoglycaemia, as well as, the proportion of patients with any severe episode of hypoglycaemia the year before was obtained. The prevalence of any form of CVD including coronary heart disease, peripheral arterial disease (including amputations) and cerebrovascular disease was evaluated. We compared the results obtained in the Group of patients with (Hypo Group) and without (Control Group) history of severe episodes of hypoglycaemia the year before entry in the registry. In addition, age, gender, duration of diabetes, BMI, HbA_{1c} , presence of any form of microvascular complication, antihypertensive and lipid treatment were also evaluated. A multivariate analysis was performed using a multiple logistic regression including CVD as dependent variable and age, gender, duration of diabetes, BMI, HbA_{1c} and the presence of severe episodes of hypoglycaemia the year before as independent variables.

Results: Repeated hypoglycaemia was an indication for starting CSII in 14.9% of patients whereas history of severe hypoglycaemia the previous year was present in 34.6% of the cases. Considering the total cohort, any manifestation of CVD was already present in the 4.7% of subjects. In Hypo Group (aged 36 ± 12 years; diabetes duration 17 ± 10 years; HbA_{1c} 8.1 ± 1.3 %; BMI 23 ± 3 kg/m²) the percentage of patients with CVD was significantly higher (6.9 %) than in the control group (3.9 %; $p < 0.05$) (aged 33 ± 12 years; diabetes duration 15 ± 9 years; HbA_{1c} 8.2 ± 1.2 %; BMI 23 ± 3 kg/m²). Previous differences in CVD percentages were not influenced by age, duration of disease, gender and the presence of antihypertensive treatment. In subjects without lipid treatment, CVD was more prevalent in Hypo group. In the multivariate analysis, age, duration of disease and gender were independent risk factors for CVD. When all age and duration of diabetes were removed from the analysis, the presence of severe hypoglycaemia emerged as an independent risk factor for CVD (β st 1.820; $p < 0.05$).

Conclusion: Data obtained from the analysis of a national registry on insulin pump therapy points to a higher prevalence of CVD in subjects with history of repeated severe hypoglycaemia. Due to the intrinsic limitations of this type of study, repeated hypoglycaemia cannot be blamed as an independent risk factor for CVD.

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Systematic evaluation of the influence of hyperglycaemia on diastolic myocardial functionU. Hoevelmann¹, L. Hermanski¹, L. Nosek¹, C. Kapitza¹, T. Heise¹, L. Heinemann¹, T. Jax^{1,2}¹Profil Institut für Stoffwechselforschung, Neuss, ²Herzzentrum Wuppertal, Universität Witten/ Herdecke, Germany.

Background and aims: Diabetes mellitus is associated with a high cardiovascular risk. There is epidemiological evidence that postprandial hyperglycemia is an important risk factor for cardiovascular events. Recent clinical-experimental studies in patients with diabetes showed that during hyperglycemia endothelial function is impaired, as is myocardial blood flow. The latter effect can be reversed by insulin in diabetic patients. The question remains, whether hyperglycemia also impairs myocardial function and whether this is

dependent of preexisting diabetes, or hyperinsulinemia. The aim of this study is therefore to evaluate the differential effects of isolated hyperglycemia in healthy and diabetic subjects on myocardial function.

Materials and methods: A total of 12 subjects were studied (4 healthy (H), 4 type 2 (T2) and 4 type 1 (T1) diabetic subjects). Each subject underwent a stepwise increase from eu- to hyperglycemic glucose levels by means of a glucose clamp for 8–12 hours (Run in with 90 mg/dl, 180, 300, and 300 mg/dl with 2.5 mU/kg/min Insulin i.v.). Repeated echocardiographic assessments of cardiac function were done at baseline and during the course of the clamp.

Results: At baseline E' (tissue Doppler) differed significantly between groups, as expected (H 13.6 ± 2.6 , T1 10.5 ± 2.9 and T2 7.6 ± 1.6 cm/s, $p < 0.05$). With increasing hyperglycemia (300mg/dl) and hyperinsulinemia for 60 min only E' in healthy subjects improved, in diabetics E' was stable at baseline levels. E/A was significantly different between H and T2 at baseline (0.94 vs. 1.46, $p < 0.05$), only T2 showed an improvement (1.2 vs. 0.94, which although not significant, could be judged as pseudonormalisation, but fell below baseline levels with additional insulin. To exclude volume effects, E/E' as a measure of LVEDP did not show relevant changes.

Conclusion: Unexpectedly hyperglycemia did not reduce but rather improve diastolic function in healthy subjects (E'), this effect was not apparent in diabetics. Pseudonormalisation was deteriorated by insulin in type 2 diabetics. Thus, healthy hearts may be better able to translate excessive glucose into cardiac work, and diabetic hearts may have lost the ability to utilize glucose, a mechanism that may not be dependent on insulin resistance but rather directly related to intracellular myocytic glucose metabolism.

Clinical Trial Registration Number: 2007-007141-13

1249

Muscular perfusion and mitochondrial oxidative activity in type 2 diabetic patients according to glycaemic control and microangiopathic complicationsS. Chiheb¹, S. Duteil², E. Cosson¹, C. Wary², J. Pariès¹, P. Carlier², P. Valensi¹¹Department of Endocrinology-Diabetology-Nutrition, Jean Verdier Hospital, AP-HP, CNRH-IdF, Paris-Nord University, Bondy, ²Institut of Myology, NMR Laboratory, F-75651, CEA, I2BM, MIRCen, IdM NMR Laboratory, UPMC University, Paris, France.

Background and aims: The aim of the study was to investigate muscle perfusion and energy metabolism in type 2 diabetic patients and to determine the respective role and interactions of chronic hyperglycaemia and microangiopathy on these alterations.

Materials and methods: We examined 96 type 2 diabetic patients distributed into 3 groups: ≤ 1 microangiopathic complication and $HbA_{1c} > 9\%$ (D1 group: $n=16$) or $HbA_{1c} < 7\%$ (D2 group: $n=56$); > 2 microangiopathic complications (D3 group: $n=24$); and 36 gender- and body mass index-matched controls (group C). Multiparametric functional Nuclear Magnetic Resonance (mpf-NMR) was performed to investigate muscular perfusion (arterial spin labeled NMR I), oxygenation (dynamic deoxy-myoglobin (dMb) ¹H NMR S) and mitochondrial function (spectroscopy ³¹P) at rest, during and after an ischemic exercise of calf muscles.

Results: In the diabetic patients, maximal post-ischemic perfusion was lower in the diabetic patients as compared to control subjects (C: 49.2 ± 3.3 ; $p=0.06$ versus D1: 40.4 ± 3.8 ml/min.100g; $p < 0.05$ versus D2: 41.4 ± 1.8 and versus D3: 37.5 ± 2.8 ml/min.100g). The oxygenation parameters were similar between groups. Mitochondrial oxidative activity was impaired in diabetic patients as compared with control subjects, with a more delayed half-time phosphocreatine recovery (C: 31.0 ± 1.8 vs D1: 42.1 ± 2.9 , D2: 36.2 ± 1.8 and D3: 37.5 ± 2.3 sec, $p < 0.05$).

Conclusion: Muscle perfusion is impaired in diabetic patients as compared to controls, especially in those with poor glycemic control and in those with multiple microangiopathic complications. Our results suggest that chronic hyperglycaemia, more than diabetic microangiopathy, affects mitochondrial function in skeletal muscle of type 2 diabetic patients.

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Alpha-lipoic acid reduces platelet reactivity in type 1 diabetic subjects

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Background and aims: A higher platelet reactivity has been associated with an increased thrombotic risk. An enhanced oxidative stress is associated with an increased TxA₂ biosynthesis and administration of supplements of vitamin E reduces oxidative stress levels and platelet reactivity. Alpha lipoic acid (ALA) is an anti-inflammatory and antioxidant molecule used in diabetic neuropathy. Aim of this study was to investigate the effect of ALA on platelet function in type I diabetic patients.

Materials and methods: We consecutively enrolled 51 type 1 diabetic subjects (mean age 44.6±10.6 years, 27 males) without any overt cardiovascular disease, who were randomized to treatment with ALA 600 mg daily (n=26) or placebo (n=25) for 4 weeks. Platelet reactivity was assessed at baseline and at follow-up by measuring the aggregation time in response to adenosine-diphosphate (ADP) + collagen with the PFA-100 method, and platelet expression of receptors glycoprotein IIb/IIIa (CD41) and P-selectin (CD62), before and after ADP stimulation (10^{-7} M), by flow cytometry.

Results: There were no significant differences in the main clinical characteristics between the two groups. At baseline, PFA-100 aggregation time and CD41 and CD62P platelet expression were similar in the two groups, both before and after ADP stimulation. After 4 weeks of treatment, ALA group showed a prolonged PFA-100 aggregation time and a lower CD41 and CD62 platelet receptor expression, both before and after ADP stimulation, compared to the placebo group (Table). Oxidative stress (8-iso PGF₂) did not change significantly in both ALA- and placebo-group.

Conclusion: Overall, these results show a reduced activation and reactivity of platelets after ALA treatment in type 1 diabetic patients. Whether these favorable effects on platelet reactivity by ALA may translate into clinical benefits would deserve assessment in large randomized clinical trials.

PFA-100 aggregation time and platelet receptor expression before and after 4 weeks of ALA

Before treatment	ALA group	Placebo group	P
Aggregation time (sec)	98 (85-106)	102 (88-107)	0.092
CD41 (MFI) - Before ADP	201 (131-236)	187 (156-228)	0.038
CD41 (MFI) - After ADP	220 (141-304)	216 (191-269)	0.150
CD62 (MFI) - Before ADP	22 (19-24)	23 (19-25)	0.130
CD62 (MFI) - After ADP	26 (24-30)	26 (23-29)	0.005
After treatment			
Aggregation time (sec)	107.5 (101-117)	97 (82-105)	<0.0001
CD41 (MFI) - Before ADP	181 (118-229)	196 (174-215)	0.0001
CD41 (MFI) - After ADP	206 (136-245)	218 (205-271)	0.0001
CD62 (MFI) - Before ADP	19 (16-24)	24 (22-26)	<0.0001
CD62 (MFI) - After ADP	25 (21-29)	29 (25-32)	<0.0001

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Initiation of insulin therapy ameliorates fibrinolysis in type 2 diabetes

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Background and aims: The prothrombotic state found in type 2 diabetes pairs hypercoagulability with impaired fibrinolysis. Both may be related to hyperglycemia-induced damage to the endothelial glycocalyx. This layer of proteoglycans and glycosaminoglycans protecting the vascular endothelium is involved in regulation of hemostasis. Our aim was to determine the effect of improved glycemic control by the initiation of insulin therapy on circulating components of the endothelial glycocalyx and markers of coagulation and fibrinolysis.

Materials and methods: In a random sample of 104 participants from the 24-week L2T3 trial in which 973 insulin-naïve patients with type 2 diabetes inadequately controlled on oral agents were randomized to insulins glargine or detemir, we measured these parameters at baseline and at the end of the study at a central laboratory. Data are expressed as medians (interquartile range) and tested non-parametrically; multivariate analysis was performed by linear regression.

Results:

Markers of coagulation and fibrinolysis	Baseline	24 Weeks	P-value
Coagulation			
Antithrombin (%)	106 (99-114)	104 (97-113)	0.059
Factor VIII (%)	71 (55-84)	65 (52-84)	0.095
Von Willebrand factor (%)	110 (84-146)	112 (83-141)	0.583
Prothrombin fragment 1+2 (pmol/l)	165 (136-218)	163 (135-194)	0.039
Coagulation/fibrinolysis			
D-dimer	0.3 (0.2-0.4)	0.3 (0.2-0.4)	0.713
Fibrinolysis			
Plasmin-antiplasmin complex (microg/l)	369 (306-466)	416 (343-515)	<0.001
Plasminogen activator inhibitor-1 (ng/ml)	100 (64-159)	87 (56-139)	0.007
Clot lysis time (min)	71 (60-85)	65 (58-77)	0.001

During the trial HbA_{1c} dropped from 8.8±0.9% to 7.2±0.9% (mean±sd) in this cohort. Markers of coagulation or glycobiology (data not shown) were not affected. However, significant changes were found in the markers of fibrinolysis. In multivariate analysis, endpoint HbA_{1c} was the most important predictor of these improvements.

Conclusion: Initiation of basal insulin therapy ameliorates the hypofibrinolytic state in type 2 diabetes, without affecting general markers of coagulation or glycobiology.

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Early detection of atherosclerosis in asymptomatic patients with type 1 diabetes

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Background: Type 1 diabetes mellitus (T1D) is associated with an increase in cardiovascular disease compared to the non-diabetic population, although there are few data on the prevention and screening of these patients. Different studies have demonstrated the functional and structural vascular changes produced in early stages of the evolution of T1D. Carotid artery intima-media thickness (cIMT) is a marker of atherosclerosis which is correlated with coronary disease. Multiple slice computerized tomography (CT) allows anatomical images of the coronary arteries to be non-invasively obtained by the quantification of calcification (calcium score).

Aim: Evaluate the presence of early atherosclerosis in asymptomatic T1D patients with an evolution of more than 10 years, with no history of ischemic or macrovascular heart disease.

Materials and methods: 90 T1D patients consecutively recruited from the outpatient clinic were studied (48 males; age: 37.2±7.6 years, 21.2±8.3 years of T1D evolution; BMI: 25.1±3.4 kg/m², HbA_{1c}: 7.9±1.0%; total cholesterol 182.9±23.1 mg/dl; HDL 58.8±14.2 mg/dl, LDL 106.9±21.6mg/dl; 64% non-smokers; 28% retinopathy; 11% microalbuminuria). Carotid ecography was performed to determine the mean cIMT (common carotid, bifurcation and right and left internal) and the presence of atheroma plaques. A high resolution multidetector CT with ECG was undertaken for calcium analysis and quantification.

Results: The mean cIMT was 0.57±0.13mm. 13/90 patients presented atheroma plaques and significantly higher HbA_{1c} (8.6±0.9% vs. 7.7±1.0% p=0.003) and a greater mean cIMT compared to patients without plaques (0.53±0.11mm. vs. 0.75±0.13mm p=0.001). 16/90 patients showed a calcium score > 0, a significantly higher age and HbA_{1c} (42.2±5.2 vs. 36.1±7.7 years p=0.003 and 8.5±0.9 vs. 7.7±1.0% p=0.004), a longer evolution of the disease

(26.0±9.4 vs. 20.2±7.8 years $p=0.02$) and a greater mean cIMT (0.53±0.11 mm. vs. 0.70±0.17 mm. $p=0.001$) compared to patients with score of 0.

Conclusion: A considerable percentage of our T1D patients with more than 10 years of disease evolution presented data suggestive of atherosclerosis, thus the inclusion of non-invasive screening methods for the detection of early atherosclerosis should be considered in routine clinical practice.

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Increased QT time and increased creatinine level are additive risk factors for mortality in patients with diabetes mellitus and peripheral arterial disease

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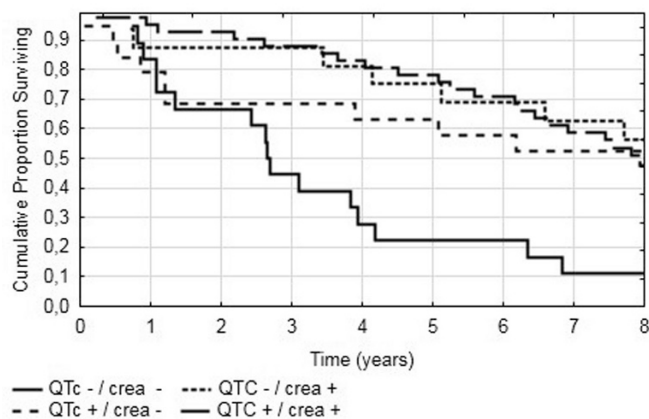
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Background and aims: Long QT-time, nephropathy and peripheral arterial disease are well established risk factors for mortality in diabetic as well as non-diabetic patients. The magnitude of combination of long QT-time and increased creatinine level as risk factor for mortality in a high risk population such as diabetic patients with lower extremity arterial disease and foot ulcer is however unknown. The aim of this study was to evaluate the significance of the combination of increased QT-time and increased creatinine level on long-term mortality in patients with diabetes and arterial disease in the lower limb.

Materials and methods: A retrospective chart study was performed including all patients with a foot ulcer and critical ischemia visiting the Diabetic Foot Clinic during one year. Peripheral ischemia was defined as previous performed vascular surgery including PTA, present ankle-brachial-index less than 0.8 or systolic arterial toe blood pressure below 50 mm Hg. Patients were divided into groups according to QTc-time \leq or $>$ 440 ms and creatinine levels of \leq or $>$ 100 (men) / \leq or $>$ 80 (men). Data are given as median and 25 and 75 percentiles. Differences between groups were evaluated using Fischer's exact test and Mann-Whitney-U-test. $P<0.05$ is considered as statistical significant.

Results: 102 patients (64 M / 38 F, 22 with type 1 diabetes / 80 with type 2 diabetes) with an age of 70 (34–80) years and a diabetes duration of 12 (0–48) years were included. Of these 8 patients with on-going dialysis were excluded. Hypertension was seen in 82 patients, heart failure in 38 and a history of myocardial infarction was present in 33 patients. At baseline HbA_{1c} was 60 mmol/l (52–71) and creatinine was 87 (71–113) μ mol/l (ref 50–100), ABI was 0.70 (0.49–0.80) and TBP was 35 (10–45) mm Hg. Group 1 consisted of patients with normal QTc interval (QTc-) and normal creatinine (crea-), group 2 of patients with normal QTc interval but high creatinine (crea+), group 3 of patients with prolonged QTc interval (QTc+) but normal creatinine and group 4 of patients with prolonged QTc interval and high creatinine level. The 8-year mortality rate was significantly higher in patients with the combination of prolonged QTc interval and pathological creatinine level ($p<0.0032$, figure 1).

Conclusion: The combination of prolonged QTc interval and pathological creatinine level is associated with increased short- and long term mortality in diabetic patients with peripheral arterial disease.



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IScore mortality prediction early after hospitalisation of an acute ischaemic stroke in diabetic patients: is this a validated and accurate prediction?

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Background and aims: Several prognostic models have been developed for evaluation of neurologic status, severity, and short-term functional outcome of stroke patients. IScore is a novel tool which has been recently developed by Canadian researchers in order to identify mortality rates within 30 days and 12 months after an ischemic stroke (IS). Since diabetic patients constitute a high risk group for IS, early prognosis is particularly important, as timely and appropriate therapeutic intervention will result in better post discharge quality care and a more judicious health decision system. The aim of the present study was to evaluate IScore mortality prediction rates in diabetic IS patients.

Materials and methods: This retrospective study was conducted in a tertiary Greek hospital. Two-hundred and sixty six ($n=266$) consecutive IS type I or type II diabetic patients, comprised the study population. Thirty day and 12 month scores were individually calculated for each patient and actual mortality was monitored at the same time intervals. IScore's predictors of mortality included older age, male sex, severe stroke, non-lacunar stroke subtype, glucose ≥ 7.5 mmol/L (135 mg/dL), history of atrial fibrillation, CAD, CHF, cancer, dementia, kidney disease on dialysis and dependency prior to the stroke. Logistic regression analysis was conducted in order to identify how accurate IScore measurements predict actual mortality. ROC curves evaluated sensitivity and specificity of IScore mortality rates.

Results: Thirty one (11.7%) out of 266 patients died 30 days after the IS. In total 41 (15.4%) patients died after 12 months. Mean (\pm SD) values for the 30 day and 12 month IScore was 176.4 \pm 42.1 and 142.6 \pm 31.5 respectively. Thirty day (HR=1.07, 95% CI: 1.04–1.09, $p<0.001$) and 12 month scores (HR=1.12, 95% CI: 1.08–1.16, $p<0.001$) were significant predictors of mortality in diabetic IS patients. A ROC curve estimated that a score of 208 (AUC=0.941, 95% CI: 0.91–0.96, $p<0.001$) has 100% (95% CI: 88.8–100) sensitivity and 83.8% (95% CI: 78.5–88.3) specificity for 30 day mortality, while a score of 169 (AUC=0.964, 95% CI: 0.93–0.99, $p<0.001$) reflects 92.6% (95% CI: 80.1–98.5) sensitivity and 92% (95% CI: 87.7–95.2) specificity for 12 month mortality. Compared to previous study in diverse population, these results exhibit higher sensitivity and specificity indicating that IScore can predict mortality more precise in diabetic patients.

Conclusion: IScore represents a well validated and accurate tool in identifying mortality early after hospitalization in acute IS diabetic patients. The identical acute clinical parameters and chronic co-morbid conditions identified within hours of admission can be used in order to predict mortality in a high-risk population after an acute stroke. In addition, IScore exhibits higher predictive accuracy specifically for diabetic patients regarding the 30 day and 12 month mortality rates.

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Screening strategy for asymptomatic coronary heart disease in Japanese subjects with type 2 diabetes

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Background and aims: There has been no consensus about the screening methods for asymptomatic coronary heart disease (CHD) in patients with diabetes. We investigated the rationale of screening methods using the treadmill tolerance test (TTT) as the first line test or the risk-guided approach including the 1998 ADA guideline for the detection of asymptomatic coronary heart disease (CHD) in Japanese patients with type 2 diabetes.

Materials and methods: Subjects included consecutive inpatients with type 2 diabetes ($n=481$). They were checked with electrocardiogram (ECG) at rest and TTT, and those with abnormal TTT findings were investigated with stress myocardial perfusion scintigraphy (MPS), and those with abnormal MPS findings were examined with coronary angiography (CAG). The number of patients who met the criteria of ADA guideline or those of patients with cardiac autonomic neuropathy (CAN) was examined. CAN was defined as the coefficients of variation of R-R intervals of ECG less than 2%.

Results: Among all subjects ($n=481$), 150 patients (31.2%) could not perform the TTT well enough for the test to be conclusive. Among the subjects who were able to complete TTT ($n=331$), a total of 69 had positive TTT, among whom, a total of 22 had positive MPS. A total of 14 subjects received CAG, and eight of them (only 1.7% of the total subject) were finally indicated to have CHD. The patients who met the criteria of ADA were 71.0% of TTT-positive patients, 68.2% of MPS-positive patients, and 75.0% of CAG-positive patients, respectively. The patients with CAN were 42.0%, 50.0% and 50.0% of patients with positive TTT, MPS and CAG, respectively. The unexpectedly low rate of asymptomatic CHD in our screening suggested that considerable subjects with asymptomatic CHD had escaped the present screening method; in fact, among those with negative results, three patients developed CHD during the follow-up. Furthermore, it was suggested that the risk-guided approach with the ADA guideline or that incorporating CAN with risk factors would have overlooked some subjects with CHD, even those who could be detected in the present screening.

Conclusion: These results suggest that neither the screening methods using TTT as the first line test nor the risk-guided approach including the ADA guideline are sufficiently adequate for detecting asymptomatic CHD in Japanese subjects with type 2 diabetes.

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Cardiovascular risk prediction is improved by adding asymptomatic coronary status to routine risk assessment in type 2 diabetic patients

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Background and aims: The aim of the study was to evaluate if silent myocardial ischemia (SMI) and silent coronary artery disease (CAD) provide significant additional value to routine cardiovascular risk assessment in type 2 diabetic (T2D) patients.

Materials and methods: We followed up to a first cardiovascular event (cardiac death, acute coronary syndrome, congestive heart failure, secondary coronary revascularization procedure, stroke, peripheral revascularization procedure and lower leg amputation) 688 (322 men, 59 ± 8 years) consecutive asymptomatic T2D patients with ≥ 1 additional risk factor who had been prospectively screened between 1992 and 2006 for SMI by stress myocardial scintigraphy and for silent CAD by coronary angiography.

Results: SMI was found in 207 (30.1%) patients and CAD in 76 of those with SMI. Ninety-eight patients had a first cardiovascular event during a 5.4 ± 3.5 [range: 0.1–19.2] year follow-up period. Cox regression analysis considering parameters predicting events but not SMI and CAD (“routine assessment”) showed in univariate analyses that macroproteinuria (hazard ratio 3.33 [1.74–6.35], $p < 0.001$), current more intensive multifactorial care (HR 0.27 [0.15–0.47], $p < 0.001$) and peripheral/carotid occlusive arterial disease (PCOAD: HR 4.33 [2.15–8.71], $p < 0.001$) independently predicted cardiovascular events. When added into the model, SMI (HR 1.76 [1.00–3.12], $p = 0.05$) and CAD (HR 2.28 [1.24–4.57], $p < 0.01$) were also independently associated with events. The cumulative probability of cardiovascular events increased with routine risk assessment (macroproteinuria and/or no current multifactorial care and/or PCOAD) and SMI: routine-/SMI- hazard ratio 1; routine+/SMI- HR 1.4 [0.6–3.5]; routine-/SMI+ HR 6.2 [3.3–11.7] and routine+/SMI+ HR 12.1 [6.4–22.5]. SMI also added to the prediction of an event in the following 5 years above and beyond routine assessment risk prediction (c statistic with or without SMI 0.788 [0.720–0.855] and 0.705 [0.616–0.794]; Hosmer-Lemeshow χ^2 5.13, $p = 0.643$ and 1.34, $p = 0.932$, respectively).

Conclusion: SMI and silent CAD are predictive of cardiovascular events in T2D patients additionally to routine risk predictors, especially represented by PCOAD, macroproteinuria and non intensive management as usually provided before 2000.

1257

Cardiovascular disease and risk factor profiles of patients with type 2 diabetes mellitus who were eligible for but did not receive a statin

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Background and aims: Statin therapy is recommended for nearly all patients with type 2 diabetes mellitus (T2DM) to reduce the risk of major cardiovascular (CV) events. Despite these recommendations, studies have shown that up to 60% of eligible patients with T2DM are untreated with statins. Therefore, it was of interest to characterize the risk factor profile of a cohort of patients with T2DM who were not on statins for 2 years despite being eligible for statin treatment.

Materials and methods: Using the General Electric electronic medical record database, patients with T2DM were included if they were ≥ 18 years of age at diagnosis and not on a statin during the baseline period (2007) and 2-year follow-up period (2008–2009). Statin eligibility was assessed using the American Diabetes Association (ADA) 2008 recommendations. CV disease (CVD) and risk factor profiles were determined by searching the database for diagnoses of specific CV-related events. Additional CV risk factor determinations were made using lipid and blood pressure (BP) measurements, obesity and smoking status, gender, and age.

Results: In a cohort of 87,351 patients eligible but untreated with statins (46% male), mean age at baseline was 60 years (92% > 40 years old) and mean HbA_{1c} was 7.1%, with 46% on antihyperglycaemic therapy. For pre-existing comorbidities and CV risk factors recorded during the 1-year baseline period, 1% of patients had overt CVD, 8% had dyslipidaemia, 76% had hypertension, 2% were smokers, and 52% were obese. For specific clinical measures at baseline, 28% had an LDL-C ≥ 100 mg/dL (2.6 mmol/L), 36% had an HDL-C below gender-specific targets, 72% had a systolic BP ≥ 140 mmHg, and 46% had diastolic BP ≥ 90 mmHg. During the 2-year follow up period without statin use, an additional 7% of patients had a new diagnosis for overt CVD, 9% for dyslipidaemia, 9% for hypertension, and 11% for obesity; and 2% started smoking.

Conclusion: Patients with T2DM eligible for, but not on statins, often have CVD and/or risk factor profiles that are suggestive of a need for CV risk reduction. Thus, a treatment gap exists between actual and optimal treatment patterns in statin-eligible patients with T2DM.

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Effects of pravastatin on coronary events and cerebral infarction in hypercholesterolaemic Japanese men with and without diabetes mellitus

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Background and aims: Since, the effect of statin was still uncertain for Japanese, whose coronary artery disease morbidity and mortality are lower than western countries, we examined the effects of pravastatin for coronary events and cerebral infarction in Japanese.

Materials and methods: The Kyushu Lipid intervention Study (KLIS) recruited 4185 men aged 45–74 years with serum total cholesterol of 220 mg/dl or greater to undertake either pravastatin or conventional treatment (including hypolipidemic drugs other than statin, probucol fibrate) and follow up for 5 years. The Cox proportional hazards model was used to calculate relative risk.

Results: At baseline, 27% of patients had a diabetes mellitus. There were 118 coronary events (acute myocardial infarction, coronary angioplasty, cardiac death) and 89 cerebral infarction in a 5-year follow-up period. Pravastatin treatment (10mg/day) was associated with 45% lower risk of coronary events in diabetes mellitus while 7% increase risk without diabetes mellitus. Pravastatin treatment was associated with 28%, 44% lower risk of cerebral infarction with and without diabetes mellitus respectively.

Conclusion: Low dose pravastatin was effective at decreasing coronary events and cerebral infarction in Japanese hypercholesterolemic men with diabetes mellitus.

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PS 117 Glycation measures and vascular risk

1259

The role of methylglyoxal in diabetic and normal cardiac ischaemia

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Background and aims: Patients with type 2 diabetes have an increased risk to develop cardiac ischemia and their response to such accidents is impaired. In the worst case, cardiac ischemia can lead to myocardial infarction, causing death to nearly 75% of diabetic population. The high concentration of glucose in diabetic cells results in increasing concentration of a secondary metabolite, the methylglyoxal (MG) that is known to have deleterious effects. We believe that prolonged exposure to MG can induce, in normal subjects, changes similar to those that occur in diabetes. Therefore, we aimed to understand the role of MG on cardiac ischemia in diabetic and normal animal model.

Materials and methods: We performed a comparative study between three groups: normal rats (W), type 2 diabetic non-obese Goto-Kakizaki rats (GK) and normal rats submitted to chronic administration of MG (WMG). Animals were sacrificed with 6 months of age, and their hearts were perfused in a Langerdorff system. At this point, hearts were submitted to different experimental conditions: control (c); ischemia (i) and ischemia-reperfusion (ir). Glycemic profile and several parameters in cardiac tissue were evaluated using mostly western blot technique.

Results: MG administration did not change glycemic profile in control animals. Comparing to W, plasmatic MG levels were higher in GK ($p<0.05$) and WMG ($p<0.05$); the cardiac MG levels were significantly higher in WMG ($p<0.001$). Looking to cardiac tissue staining of AGEs (Advanced Glycation End Products) and their receptors (RAGEs), it was observed an increasing fluorescence in GK and WMG groups. JNK protein expression particularly demonstrated a decreasing in (i)-subgroups among the three groups ($W>GK>WMG$); levels in WMG(ir) rats were also decreased ($p<0.001$) in comparison to W(ir). Phospho-JNK expression increased only in W(i) and GK(i) ($p<0.001$; $p<0.05$), comparing to their controls. All (ir)-subgroups showed P-JNK expression lower than their respective (c)-subgroups ($p<0.05$). Regarding to Akt protein, it levels decreased generally in (i)- and (ir)-subgroups among three groups. Levels of GK and WMG (c)-subgroups decreased in relation to W ($p<0.001$); the (i)-subgroups levels also decreased among the three groups ($p<0.01$; $p<0.05$), and (ir)-subgroup levels were only significantly lower in WMG ($p<0.05$). In phosphorylated form of Akt, it was observed decreasing levels in GK and WMG, when compared to W group: control ($p<0.001$; $p<0.01$); ischemic ($p<0.01$) and ischemic-reperfusion ($p<0.05$; $p<0.01$) subgroups. The ratio Bcl-2/Bax showed noteworthy an increase in W(i) and GK(i) comparing to their controls ($p<0.01$; $p<0.05$). WMG(i) showed a less increased ratio that was significantly lower than W(i) and GK(i) with $p<0.01$. Relating to W(c), GK and WMC (c)-subgroups decreased their values ($p<0.05$). Levels of Caspase 3 increased significantly in W(ir) and GK(ir) ($p<0.05$), comparing respectively with W(c) and GK(c). GK(ir) and WMG(ir) levels were higher than W(ir) ($p<0.05$).

Conclusion: The administration of methylglyoxal in normal rats is able to mimic the profile founded in diabetic rats and even some parameters are more impaired. Our results re-affirm the toxicity of the compound and demonstrate the potentiality of methylglyoxal to be a therapeutic target in diabetic cardiac ischemia disease.

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1260

Vascular actions of methylglyoxal with implications for endothelial dysfunction

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Background and aims: Modern diets can cause modern diseases. Research has linked a metabolite of sugar, methylglyoxal (MG), to the development of diabetic complications, but the exact mechanism has not been fully elucidated.

The present study was designed to investigate whether MG could directly influence endothelial function, oxidative stress and inflammation in Wistar and Goto-kakizaki (GK) rats, an animal model of type 2 diabetes.

Materials and methods: Wistar and GK rats treated with MG in the drinking water for 3 months were compared with the respectively control rats. The effects of MG were investigated on NO-dependent vasorelaxation in isolated rat aortic arteries from the different groups. Insulin resistance, NO bioavailability, glycation, a pro-inflammatory biomarker monocyte chemoattractant protein-1 (MCP-1) and vascular oxidative stress were also evaluated.

Results: Methylglyoxal treated Wistar rats significantly reduced the efficacy of NO-dependent vasorelaxation ($p<0.001$). This impairment was accompanied by a threefold increase in the oxidative stress marker nitrotyrosine. Advanced glycation endproducts (AGEs) formation was significantly increased as well as MCP-1 and the expression of the receptor for AGEs (RAGE). NO bioavailability was significantly attenuated and accompanied by an increase in superoxide anion immunofluorescence. Methylglyoxal treated GK rats significantly aggravated endothelial dysfunction, oxidative stress, AGEs accumulation and diminished NO bioavailability when compared with control GK rats.

Conclusion: These results indicate that methylglyoxal induced endothelial dysfunction in normal Wistar rats and aggravated the endothelial dysfunction present in GK rats. The mechanism is at least in part by increasing oxidative stress and/or AGEs formation with a concomitant increment of inflammation and a decrement in NO bioavailability. The present study provides further evidence for methylglyoxal as one of the causative factors in the pathogenesis of atherosclerosis and development of macrovascular diabetic complication.

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1261

Advanced glycation end-products-induced vascular calcification is mediated by oxidative stress: functional roles of NAD(P)H-oxidase

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Background and aims: Vascular calcification is associated with increased morbidity and mortality in patients with diabetes mellitus and end-stage kidney disease. Especially, medial artery calcification, known as Monckeberg's type of calcification, is often detected in these patients. Growing evidence suggests that advanced glycation end-products (AGEs) are associated with vascular calcification, although their actions are still unclear. On the other hand, oxidative stress leads to vascular damage through diverse mechanisms. Thus, we investigated an *in vitro* study to elucidate the effects of AGEs and the roles of NAD(P)H oxidase in the pathogenesis of vascular calcification.

Materials and methods: Rat vascular smooth muscle cells (A7r5) were incubated in calcification medium with AGE3 to measure calcium deposition, to evaluate apoptosis (ELISA) and to determine mRNA expression levels of osteopontin (OPN), osteocalcin (OC), Runx2, Nox-1, Nox-4, and p22phox (real-time PCR). AGE3 was prepared by incubating albumin with 0.1M glycolaldehyde and 5mM diethylenetriaminepentaacetic acid in 0.2M phosphate buffer (pH7.4) at 37°C for 7 days. Nox-1, Nox-4, and p22phox mRNAs were knocked down by using small interfering RNA (siRNA) technique.

Results: Calcium deposition was increased by AGE3 in a dose- (100-300µg/dl) and time- (3-7days) dependent manner in A7r5 cells. AGE3 was the most potent in calcium deposition among AGEs, which we had examined. Expression levels of the OPN, OC, and Runx2 mRNAs were significantly higher in AGE3 treatment than those in control BSA treatment, indicating the osteoblastic transdifferentiation of the vascular smooth muscle cells. In addition, apoptosis was enhanced within 24hrs in cells treated with AGE3, compared to that with control BSA. Increased expressions of Nox-1, Nox-4, and p22phox mRNAs (3 to 6 folds) were also observed in cells treated with AGE3, suggesting that AGEs most probably promote reactive oxidative species production resulting in the progression of calcium deposition. Therefore, we examined the effects of silencing of these mRNAs by using siRNA transfection method. We found that AGE3-stimulated calcium deposition was significantly decreased in the cells transfected by either Nox-4 or p22phox siRNA, which effectively depressed their mRNA levels, compared to the controls (scramble). In contrast, no significant effect was shown in silencing of Nox-1, although its mRNA level was effectively depressed to less than 5%.

Conclusion: The present findings indicate the involvement of osteoblastic transdifferentiation of the vascular smooth muscle cells and these apoptosis as well as oxidative stress in the pathogenesis of AGEs-induced vascular calci-

fication. Our findings also suggest that activated NAD(P)H oxidase, which is induced by increased expression of Nox-4 and p22phox, may play important roles. These molecules could be a target of new strategy to prevent vascular calcification as well as vascular damage.

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Endothelial progenitor cells and glycated albumin level are correlated with atherogenesis in type 2 diabetic patients

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Background and aims: We investigated the relationship between endothelial progenitor cells (EPCs) and atherosclerotic plaque formation in type 2 diabetic patients without documented ischemic disease.

Materials and methods: We conducted a clinic-based, prospective study of type 2 diabetic patients. A total of 73 subjects (38 men and 35 women; mean age, 56.2 ± 7.7 years) were enrolled after cardiac MRI and ankle-brachial index assessments to exclude patients with ischemic disease. Intima media thickness and plaques in the carotid artery were measured by ultrasonography. Numbers of circulating EPCs (CD34⁺/CD133⁺/CD309⁺ cells) were enumerated by flow cytometry.

Results: Compared to subjects without carotid artery plaques, patients with plaques were significantly older (52.3 ± 8.0 vs. 57.8 ± 7.1 years, $p = 0.006$) and had decreased EPC levels (412 (349) vs. 279 (354), $p = 0.027$). Serum glycated albumin (GA) level and GA/HbA1c ratio tended to be lower in patients with plaques ($p = 0.091$ and 0.067 , respectively). Binary logistic regression analysis revealed that old age, a low EPC level, and a high serum GA level were independently correlated with carotid artery plaque formation. Most subjects with a lower EPC count and a higher GA/HbA1c ratio than the medians of the respective parameters had carotid artery plaques (Fig.1).

Conclusion: EPCs and serum GA level could be sensitive biomarkers indicating endothelial protection and damage for predicting atherogenesis in type 2 diabetic patients.

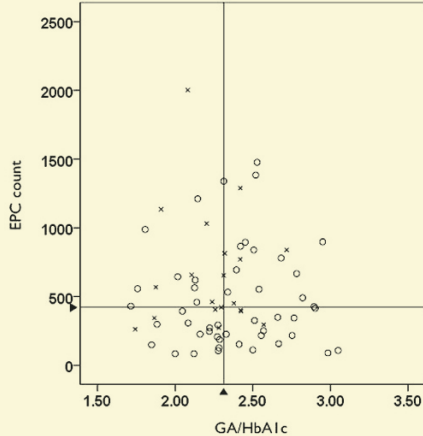


Figure 1. Scatter plot showing the distribution of subjects with and without carotid artery plaque formation according to serum GA/HbA1c and EPC levels. Carotid artery plaque formation was assessed by ultrasonography. GA = glycated albumin; EPC = endothelial progenitor cells; The symbol "●" indicates the medians of the respective parameters; The symbol "○" indicates the patients with carotid plaques; The symbol "×" indicates the patients without carotid plaques.

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Increased plasma levels of the methylglyoxal-derived advanced glycation end-product tetrahydropyrimidine in type 1 diabetes and in atherosclerotic plaques: association with soluble vascular cell adhesion molecule 1

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Background and aims: Methylglyoxal (MGO) is a major precursor in the formation of Advanced Glycation Endproducts (AGEs). MGO primarily reacts with arginine to three MGO-derived AGEs: 5-hydro-5-methylimidazolone, argpyrimidine and tetrahydropyrimidine (THP). MGO-derived AGEs are implicated in the development of vascular complications. We studied, with the use of a novel antibody, whether THP is elevated in type 1 diabetes mellitus (T1DM) and related to endothelial dysfunction, low-grade inflammation and atherosclerosis.

Materials and methods: We raised and characterized a monoclonal antibody against the MGO-derived AGE THP and developed a competitive ELISA. THP was measured in 196 T1DM subjects and 195 controls. We measured plasma markers of endothelial activation, i.e. soluble vascular cell adhesion molecule 1 (sVCAM-1) and Von Willebrand factor (vWF), and low-grade inflammation, i.e. high-sensitivity C-Reactive Protein (hsCRP). In addition, we performed immunohistochemical analyses to determine the presence of MGO-derived THP and the major AGE N^ε-(Carboxymethyl)lysine (CML) in atherosclerotic arteries.

Results: The raised antibody was specific for the MGO-derived AGE THP. Plasma levels of THP were higher in subjects with T1DM than in control subjects with median and IQR of 115.5 U/ μ l [102.4 - 133.2] and 109.8 U/ μ l [91.8 - 122.3], respectively ($p=0.03$). In regression analyses sVCAM-1 was significantly associated with plasma THP, independent of possible confounders. Per SD increase in THP, sVCAM-1 increased by 0.47 SD ($p<0.001$). THP was not associated with vWF, nor with hsCRP. We found no association of THP with microvascular complications. THP, as well as CML, was detected in atherosclerotic arteries in extracellular lipids, macrophages and calcification sites, including surrounding smooth muscle cells. CML was also present in the endothelium and adventitial blood vessels, while THP was not.

Conclusion: Plasma levels of the MGO-derived THP are significantly associated with diabetes and correlated with sVCAM-1; i.e. an endothelial marker associated with atherosclerosis development. THP and CML were found in atherosclerotic arteries. Our results are consistent with a proposed pathophysiological role of the MGO-derived AGE THP in vascular complications in patients with and without diabetes.

Supported by: CTMM; NHF; DDRF and DKF. PREDICCT.

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Advanced glycation endproducts in atherosclerotic plaques: no further increase in patients with diabetes

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Background and aims: Atherosclerosis is the major underlying cause of morbidity and mortality in patients with diabetes. Advanced glycation endproducts (AGEs) are formed when concentrations of reducing sugars and reactive oxygen species increase. AGEs lead to vascular damage through alteration of protein function and interaction with their receptors. Therefore, AGEs may explain the increased risk of atherosclerotic events in diabetes. The aim of this study was to determine whether concentrations of AGEs in atherosclerotic plaques are higher in patients with diabetes.

Materials and methods: 75 plaque homogenates were randomly selected from the Athero-Express cohort, containing patients undergoing carotid endarterectomy (48% diabetes, 72% male, mean age: 67.7 ± 8.8 years). Presence of diabetes was determined on the basis of a questionnaire. The concentrations of the protein-bound AGEs N^ε-(carboxymethyl)lysine (CML),

N^ε-(carboxyethyl)lysine (CEL) and 5-hydro-5-methylimidazolone (MG-H1) in homogenates were quantified by UPLC tandem mass spectrometry. Plaque concentrations of inflammatory molecules interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) were measured using a multiplex suspension array system. Plaques were histologically scored for lipid content. In a subset of 40 plaques, immunohistochemistry was performed with antibodies against CML and MG-H1. Data were presented as median and interquartile range and analyzed using ANOVA, after log-normalization. Correlations were calculated using the spearman method.

Results: Concentrations of AGEs in homogenates of carotid plaques of individuals without- and with diabetes did not differ significantly: CML 79.0 (58.6–126.3) vs. 90.6 (65.6–150.1) nmol/mmol lysine, $p=0.23$, CEL 89.2 (82.0–106.9) vs. 93.4 (75.7–109.0) nmol/mmol lysine, $p=0.97$ and MG-H1 367.0 (307.0–866.7) versus 406.2 (288.2–660.0) nmol/mmol lysine, $p=0.95$. Levels of MG-H1 were higher in plaques with increased fatness; no fat: 323.7 (272.9–511.4), <40% fat: 372.1 (307.4–640.9), >40% fat 445.3 (302.1–957.1) nmol/mmol lysine, $p_{\text{trend}}=0.04$. A similar, but non-significant, trend was also observed for CML, but not for CEL. Within plaques, extensive positive staining for MG-H1 and CML was present in macrophages and endothelial cells. Additionally, a correlation between MG-H1 with IL-8 ($r=0.334$, $p<0.01$) and MCP-1 ($r=0.256$, $p=0.03$) was found. This was also true for CML ($r=0.346$, $p<0.01$ and $r=0.249$, $p=0.03$, respectively). CEL was correlated with MCP-1 ($r=0.291$, $p=0.01$), but not IL-8 (0.135 , $p=0.265$).

Conclusion: The concentrations of CML, CEL and MG-H1 in atherosclerotic plaques are not further increased in patients with diabetes, suggesting that AGE formation in plaques is associated with localized plaque metabolism and inflammation, rather than with a generalized increase in metabolism due to diabetes.

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Glycated albumin to glycated haemoglobin ratio (GA/HbA_{1c}) is associated with the presence of carotid plaque in patients with type 2 diabetes mellitus

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Background and aims: Epidemiological evidence indicates that postprandial hyperglycemia is a risk factor for cardiovascular disease. Because of the shorter half-life of serum albumin compared to erythrocytes, serum glycated albumin (GA) level reflects shorter-term glycemic control status compared to HbA_{1c}. Several reports also indicated that serum GA reflects postprandial blood glucose excursions better than HbA_{1c}. Although GA (%) to HbA_{1c} (%) ratio (GA/HbA_{1c}) is generally estimated approximately 3, there is a certain range (typically 2–4) in the distribution of GA/HbA_{1c} value in patients with type 2 diabetes mellitus (T2DM). In this study, we assessed the relationship of GA/HbA_{1c} with other conventional glycemic control parameters and evaluated the potential clinical usefulness of the measurement of GA/HbA_{1c} for predicting atherosclerotic cardiovascular outcomes in T2DM.

Materials and methods: Of the patients with T2DM admitted to our hospital for glycemic control during 2005–2009, 148 patients (99 males and 49 females; 56±13 years old) were examined in this study. Exclusion criteria included endocrine complications, steroid treatment, ketosis, malignant disease, anemia, and other systemic diseases. Clinical measurements including fasting plasma glucose (FPG), GA, HbA_{1c}, and lipid profiles were taken on admission. HbA_{1c} value in Japan Diabetes Society unit (%) was used for statistical analysis. Carotid Intima-media thickness (IMT) and the presence of carotid plaque were assessed by B-mode ultrasonography performed bilaterally on common carotid artery, carotid bifurcation, and internal carotid artery. The presence of carotid plaque was defined as focal IMT ≥1.0 mm with marked protuberance. **Results:** GA/HbA_{1c} was positively correlated with age ($P<0.0001$), duration of T2DM ($P=0.0008$), FPG ($P<0.0001$), GA ($P<0.0001$), and HbA_{1c} ($P=0.0043$). Carotid plaque was presented in 85 patients, whereas 63 patients were without carotid plaque. In patients with carotid plaque, GA and GA/HbA_{1c} were higher than those without carotid plaque (GA, $P=0.015$; GA/HbA_{1c}, $P=0.0003$). On the other hand, neither FPG nor HbA_{1c} was significantly different in both groups (FPG, $P=0.49$; HbA_{1c}, $P=0.76$). The results of logistic regression analysis showed that GA/HbA_{1c} was significantly associated with the presence of carotid plaque (age- and sex-adjusted OR, 2.55; 95% CI, 1.11–6.42; $P=0.035$).

Conclusion: The positive correlation of GA/HbA_{1c} with other glycemic control parameters suggested that higher GA/HbA_{1c} value reflects worse blood glucose control. GA/HbA_{1c} value was higher in the patients with carotid plaque and was emerged as a strong predictor of the presence of carotid plaque in multivariable logistic regression analysis. Thus, the measurement of GA/HbA_{1c} may be useful to predict the risk of atherosclerotic cardiovascular events in T2DM.

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Soluble RAGE plasma levels are inversely associated to severity of coronary atherosclerosis and are genetically influenced

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Background and aims: Hyperglycemia is associated with increased production of AGEs (advanced glycation end-products). RAGE [receptor for AGEs] plays an important role in the development and progression of vascular disease while circulating soluble RAGE (sRAGE), acting as decoy for RAGE ligands has a protective function against vascular complications. Additionally, genetic variants of the RAGE gene have been associated with individual differences in sRAGE concentration and vascular disease risk. The aim was to examine the association between sRAGE and a functional single-nucleotide polymorphism (SNP) in the RAGE gene (Gly82Ser; rs2070600), a SNP associated with increased ligand affinity of RAGE, with severity of coronary artery disease (CAD) in patients with stable angina.

Materials and methods: A total of 277 patients (175 males, 102 with T2DM, 65.2±10.1 years) with stable angina were studied. Serum sRAGE was measured by ELISA and the Gly82Ser polymorphism (determined in a subgroup of 127 patients) was determined with a method that combined polymerase chain reaction and sequence-specific oligonucleotide probes with array technology.

Results: Patients with diseased vessels ≥2 (n=95) had significantly lower levels of sRAGE than patients with diseased vessels ≤1 (n=182) [272 (178–456) pg/mL median (interquartile range) and 518 (228–968) pg/mL respectively, $P<0.0001$] independently of T2DM. In multivariable logistic regression analysis after adjustment for potential confounders, the sRAGE levels were significantly and independently associated with severity of CAD (OR = 0.672, 95% CI=0.474–0.951, $P=0.025$). Genetic analysis revealed 4.2 % of patients with GS genotype and 95.8 % of patients with GG genotype. The RAGE GS genotype distribution did not differ between patients with diseased vessels ≤1 (5%) and those with diseased vessels ≥2 (3%) while showed an association with sRAGE levels. Specifically, subjects with GS genotype had lower sRAGE levels versus those with the common GG genotype of RAGE [184 (62–234) pg/mL and 300 (181–427) pg/mL respectively, $P=0.0021$]. RAGE Gly82Ser polymorphism ($\beta = -0.253$, $P=.0026$) and creatinine ($\beta = 0.231$, $P=.0099$) were major factors affecting sRAGE concentrations.

Conclusion: Circulating sRAGE levels are inversely associated to severity of CAD and the lowest sRAGE levels are genetically influenced. However RAGE Gly82Ser polymorphism does not seem to be involved in genetic susceptibility to CAD severity.

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Lack of association between skin autofluorescence level and intermediate markers of cardiovascular risk in patients with type 2 diabetes and metabolic syndrome

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Background and aims: The AGE Reader is supposed to provide a non invasive determination of the accumulation of advanced glycation end products (AGE) in the skin. Consequently this measurement should be related to microvascular and cardiovascular complications more especially as AGE level was previously reported associated with ischemic cardiovascular mortality in type 2 diabetes. Consequently we hypothesized that skin autofluorescence might be related to carotid intima-media thickness (IMTc) and pulse wave velocity (PWV).

Materials and methods: We studied 22 type 2 diabetic patients, 62±8 years old (group A), 25 type 1 diabetic patients 50±9 (group B), 20 patients with

metabolic syndrome, 55 ± 10 (group C), and 43 control subjects, 49 ± 8 (group D). Age, body mass index, blood pressure, duration of diabetes, microvascular and cardiovascular complications were recorded. HbA1C level. PWV (by pulse pen), IMTc and AGE levels (AGE Reader) were measured for each patient.

Results: AGE levels were mildly correlated with age ($r^2=0.15$, $p<0.001$). A mild increase in AGE level was observed only in group A (2.82 ± 0.55 AU) and B (2.59 ± 0.57 AU) as compared with group C (2.26 ± 0.63 AU) and D (2.21 ± 0.50 AU) (ANOVA $p<0.05$). In the patients with microvascular complications, the AGE level was 12.6 % higher versus patients without microvascular complications ($p=0.04$, ns after adjustment for age). Overall a weak correlation was observed between AGE and HbA1C levels ($r^2=0.13$, $p<0.001$). No correlation was observed between AGE level and either PWV or IMTc measurements or the duration of diabetes. Adjustment of individual AGE level for age did not change the findings.

Conclusion: In our study, Skin AGE level assessed by AGE Reader was only found correlated with the age of patients but not with microvascular and cardiovascular complications. These findings raise the question of the added value of skin AGE level measurement in the assessment of the mechanisms of macroangiopathy.

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Skin autofluorescence and the presence of microvascular and macrovascular complications in patients with type 2 diabetes mellitus: a multicenter study

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Background and aims: Skin autofluorescence (SAF) is a non-invasive marker of accumulation of advanced glycation end products. SAF has been shown to correlate strongly with and to be predictive for both microvascular and macrovascular complications in patients with type 2 diabetes in a single-center primary care cohort. The present cross-sectional study aims to confirm the association between SAF and microvascular and macrovascular complications in patients with type 2 diabetes mellitus in a multicenter secondary care setting.

Materials and methods: We analysed 566 subjects with type 2 diabetes mellitus followed in hospital-based outpatient clinics in 5 Dutch hospitals. Clinical data including micro- and macrovascular complications were gathered.

Results: Median age was 64 years, median duration of diabetes 13 years and median HbA1c 58 mmol/mol. 58% of the patients had microvascular complications (38% had nephropathy, 36% retinopathy, 36% neuropathy), and 42% had macrovascular complications. The median UKPDS 10 years risk for coronary events was 33%. Median SAF was 2.77 which is elevated compared to age-matched healthy controls (median SAF 2.46). More specifically, SAF levels were increased in subjects with diabetic micro- and/or macrovascular complications as compared to the subjects without diabetic complications (median SAF 2.98 for both micro- and macrovascular complications versus 2.84 for macrovascular, 2.79 for microvascular and 2.56 without complications, $p<0.001$). Multivariate analysis showed that the independent determinants of the presence of macrovascular complications were age, serum creatinine, SAF, gender and duration of diabetes. In another model, the level of SAF was determined by age, smoking, serum creatinine, systolic blood pressure and presence of microvascular complications and/or macrovascular complications.

Conclusion: This study confirms that SAF is increased in type 2 diabetic patients, particularly in those with microvascular and macrovascular complications, in a multicenter secondary care setting.

PS 118 Central and ectopic fat

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GFT505, a dual PPAR α / δ agonist has beneficial effects in animal models of NAFLD/NASH through PPAR α -dependent and independent mechanisms

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Peroxisome Proliferator-Activated Receptors (PPARs) α and δ are important regulators of lipid and glucose metabolism and inflammation. GFT505, a dual PPAR α / δ agonist, reduces plasma triglycerides and LDL-cholesterol, increases HDL-cholesterol, improves insulin sensitivity and lowers inflammatory markers in patients with pre-diabetes. In addition, GFT505 improves liver function markers like alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (γ GT). In rodents after oral administration, GFT505 concentrates in the liver with limited extra-hepatic exposures due to an extensive entero-hepatic cycle. Hence, GFT505 may be an interesting candidate to treat non-alcoholic fatty liver diseases (NAFLD) and steato-hepatitis (NASH). We report here on studies assessing the potential of GFT505 in animal models of NAFLD/NASH. Experimental NAFLD/NASH was induced by a 7-week methionine-choline deficient diet (MCDD) in db/db mice. Compared to the control diet, MCDD provoked a large intra-hepatic triglyceride accumulation. At histological examination, a marked macrovesicular and moderate microvesicular steatosis was accompanied by increased inflammation and eosinophilic cytoplasmic inclusions consistent with Mallory bodies. No sign of fibrosis was detected. In MCDD mice concomitantly treated with GFT505 (30 mg/kg/d per os), intra-hepatic triglyceride content was comparable to that measured in mice fed a control diet. Microscopic examination showed that GFT505 administration completely prevented the MCDD induced macrovesicular steatosis. Inflammatory cell foci and eosinophilic inclusions were no longer observed. Consistent with the liver protection by GFT505, plasma ALT activity was comparable in GFT505+MCDD and control groups while it was 3–4 fold higher in mice fed MCDD alone. Quantitative PCR experiments showed that the MCDD-induced rise in expression of inflammatory and pro-fibrosis genes (IL1 β , TNF α , TGF β , collagens) was blocked by GFT505. All these effects of GFT505 were also observed in C57Bl/6 mice fed a MCDD. To assess the role of PPAR δ activation in the beneficial effects of GFT505, dyslipidemic ApoE2-KI mice deficient for PPAR α expression were fed a high fat diet with or without GFT505 (30 mg/kg/d per os) for 6 weeks. Compared to the control group, GFT505 treatment reduced intra-hepatic triglycerides. This correlated with reduced micro- and macrovacuoles and lower cellularity in sinusoids (Kupffer cells). Furthermore, GFT505 treatment significantly reduced expression of inflammatory and pro-fibrosis genes like IL1 β , TNF α , TGF β and collagens. Taken together with the clinical data, these pre-clinical studies argue for the potential of a mixed PPAR α / δ agonist like GFT505 in the prevention or treatment of NAFLD/NASH. These results highlight the role of PPAR δ activation in the hepatic protection provided by GFT505.

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Increased dietary polyunsaturated fatty acid is significantly associated with a lower prevalence of steatosis in patients with type 2 diabetes

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is commonly associated with obesity, metabolic syndrome and type 2 diabetes. Although dietary fat is an important contributor of liver fat accumulation, the role of individual fatty acid in the development of liver fat content is unclear. In this study, we set out to determine whether liver fat content (LFC), was associated with red blood fatty acid composition in people with type 2 diabetes. **Materials and methods:** 162 type 2 diabetic patients were included in this study. LFC was measured using ¹H-MR Spectroscopy; hepatic steatosis was defined as LFC $\geq 5.5\%$. Red blood fatty acid composition was measured by chromatography. Erythrocyte fatty acid composition reflects recent dietary intake (approximately the past 2 months). We also evaluated the saturation index (SI) by the ratio of saturated to monounsaturated fatty acid (palmitic acid to palmitoleic acid). The SI reflects the activity of several genes involved

in lipid metabolism such as stearoyl coenzyme -A desaturase. Fatty acid composition was reported as the percentage by weight of the total fatty acids in the erythrocytes membrane. The fatty acids compositions were categorized into quartiles. Logistic regression models were used to calculate odds ratios as estimates of the relative risks for steatosis associated with each quartile of fatty acids. Age, BMI and gender were included in the multivariate analysis.

Results: 109 (67.2%) patients had steatosis. Patients with steatosis had a higher BMI ($p = 0.0005$), higher plasma ALAT levels ($p < 0.001$), and higher plasma triglyceride levels ($p = 0.009$) than did patients without steatosis. We report a significant association between palmitic acid (16:0), palmitoleic acid (16:1n-7) concentrations and higher liver fat content. Total polyunsaturated fatty acid (PUFA), homo- γ -linolenic acid (20:3n-6), docosahexaenoic acid (22:6n-3), arachidonic acid (20:4 n-6) were associated with lower liver fat content. The SI was significantly associated with higher liver fat content.

Conclusion: The increased SI and palmitoleic acid concentration in subject with steatosis suggests that stearoyl-coA desaturase activity and lipogenesis were increased in these patients. Our data showed that an increased dietary PUFA is significantly associated with a lower prevalence of steatosis in patients with type 2 diabetes. These results suggest that PUFA supplementation could be a promising treatment for NAFLD in patient with type 2 diabetes.

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The effect of SFN on hepatic lipogenesis and fibrosis

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Background and aims: Sterol regulatory binding protein-1c (SREBP-1c) and transforming growth factor (TGF)- β /Smad signaling pathways are master regulators of lipogenic and fibrogenic gene expression and promote hepatic steatosis and fibrosis, respectively which are closely associated with insulin resistance. Suforaphane (SFN), an abundant isothiocyanate found in cruciferous vegetables is a potent activator of anti-oxidant Nrf2 protein. However, the effects of SFN on hepatic lipogenesis and fibrosis have not been investigated. Here, we examined whether SFN regulates SREBP-1c expression and TGF- β /Smad signaling pathway in liver.

Materials and methods: Northern and Western blot analyses were performed to examine the potential effects of SFN and Ad-Nrf2 on expression of SREBP-1c, collagen and fibronectin in liver cell lines (H4IIE, HepG2, AML12). Transient transfection study was performed to assess the effect of SFN and Nrf2 on SREBP-1c promoter activity and (CAGA)₃ MLP-Luc, a synthetic TGF- β /Smad3 responsive reporter. The effect of Ad-Nrf2 on LXR-DNA binding activity was determined by electrophoretic mobility shift assay. The in vivo effects of SFN and Ad-Nrf2 on hepatic lipogenesis and fibrosis were verified in high fat diet (HFD)-fed mice and in bile duct ligated (BDL) mice, respectively. **Results:** Overexpression of Nrf2 and SFN inhibited insulin or LXR agonist-stimulated SREBP-1c expression and its promoter activity. SFN also inhibited TGF- β -stimulated collagen and fibronectin expression. Nrf2 also inhibited LXR-agonist-stimulated LXR-DNA binding to its response consensus element on SREBP-1c promoter. Moreover, tail vein injection of Ad-Nrf2 in HFD-fed mice inhibited hepatic steatosis through inhibition of SREBP-1c expression. SFN administration also reduced BDL-induced hepatic fibrosis.

Conclusion: This study shows that SFN exerts a protective effect against hepatic lipogenesis and fibrosis through downregulation of SREBP1c and TGF- β -induced ECM proteins.

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Increased selenoprotein P levels in subjects with visceral obesity and non-alcoholic fatty liver disease

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Background and aims: Selenoprotein P (SeP) has recently been reported as a novel hepatokine that regulates insulin resistance and systemic energy metabolism in rodents and humans. We explored the associations between SeP, visceral obesity, and non-alcoholic fatty liver disease (NAFLD).

Materials and methods: We examined serum SeP concentrations in subjects with increased visceral fat area (VFA) or liver fat accumulation measured

using computed tomography (CT). Our study subjects included 120 non-diabetic healthy individuals. In addition, we evaluated the relationship between SeP and cardiometabolic risk factors, including homeostasis model of insulin resistance (HOMA-IR), high sensitivity C-reactive protein (hsCRP), adiponectin values, and brachial-ankle pulse wave velocity (baPWV).

Results: Subjects with NAFLD showed increased levels of HOMA-IR, hsCRP, VFA, and several components of metabolic syndrome and decreased levels of adiponectin and HDL-cholesterol compared to those of controls. Serum SeP levels were positively related to VFA, hsCRP, and baPWV and negatively related to the liver attenuation index. Not only subjects with visceral obesity but also those with NAFLD exhibited significantly increased SeP levels ($P < 0.001$). In multiple logistic regression analysis, the subjects in the highest SeP tertile showed a higher risk for NAFLD compared to those in the lowest SeP tertile, even after adjusting for potential confounding factors (odds ratio = 7.48, 95% CI = 1.72–32.60, $P = 0.007$).

Conclusion: Circulating SeP levels are increased in subjects with NAFLD as well as in those with visceral obesity and may be a novel biomarker for NAFLD.

Multiple Logistic Regression Analysis with Non-Alcoholic Fatty Liver Disease as a Dependent Variable

	T1	T2	p	T3	p
	(OR, 95% CI)			(OR, 95% CI)	
Unadjusted	1.00	5.75 (2.11, 15.69)	0.001	10.55 (3.73, 29.84)	<0.001
Model 1	1.00	5.56 (1.98, 15.57)	0.001	10.48 (3.69, 29.75)	<0.001
Model 2	1.00	5.54 (1.76, 16.76)	0.003	5.68 (1.78, 18.10)	0.003
Model 3	1.00	4.78 (1.42, 16.10)	0.011	5.23 (1.52, 18.03)	0.009
Model 4	1.00	6.30 (1.51, 26.28)	0.012	7.48 (1.72, 32.60)	0.007

Model 1: Adjusted for age, sex

Model 2: Adjusted for age, sex, BMI, and smoking status

Model 3: Adjusted for age, sex, BMI, smoking status, SBP, DBP, triglycerides, and HDL-cholesterol values

Model 4: Adjusted for age, sex, BMI, smoking status, SBP, DBP, triglycerides, HDL-cholesterol, hsCRP, adiponectin, and HOMA-IR values

Clinical Trial Registration Number: NCT01257685

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Visceral adipose tissue-derived serine protease inhibitor is not different in patients with atherosclerosis or diabetes but is associated with severity of peripheral arterial disease

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Background and aims: Visceral adipose tissue-derived serine protease inhibitor (Vaspin), a unique insulin-sensitizing adipocytokine in obesity, has been attributed to be involved in the development of insulin-resistance mediated type 2 diabetes mellitus (T2DM) as well as consecutive atherosclerosis. Even more, a T2DM independent effect on the ontogeny of atherosclerosis was suspected. However, evidence for Vaspin's role in atherosclerosis is scarce and conflicting. Thus we investigated Vaspin levels in patients with different disturbances of glucose metabolism and systemic atherosclerosis (SA) as well as in age- and gender matched controls (CO).

Materials and methods: We investigated Vaspin values in 234 SA patients (66±10 years, 49% female, 30% normal glucose tolerance (NGT), 35% pre-diabetes (PRE), 36% T2DM) and 66 CO (65±11 years, 55% female) by ELISA (BioVendor, Modrice, Czech Republic). Statistics (SPSS 18.0) included KS-Test, t-test, χ^2 -Test, ANOVA and uni/multivariate regression, as appropriate. Non-parametric variables were log10-transformed for normal distribution. Data are given as median (25;75 percentile). A p-value <0.05 was significant.

Results: In contrast to previous smaller studies, Vaspin did not differ between SA and CO patients (181 (120;345) vs 237 (129;339) ng/l, $p=0.256$). In addition, further analysis revealed no difference between the groups CO, SA-NGT, SA-PRE, and SA-T2DM. We observed a significant difference in gender: female 244 (138;397) vs male 176 (113;259) ng/l, $p=0.002$. Vaspin was not associated with levels of thyroid hormones such as Thyroid-Stimulating-Hormone, Free T3, and Free T4. In order to exclude overestimation of as-

sociations in our cohort at highest possible cardiovascular risk, we divided patients (SA) in three Vaspin Tertiles. In all SA patients Vaspin Tertiles were associated with age, fasting insulin, aspartate-amino-transferase, gamma-glutamyl-transferase, gender, stage of peripheral arterial disease (PAD), smoking, and medication with beta-blockers, but not with history or stage of coronary heart disease, stroke, medications with other anti-hypertensive drugs, anti-lipidemic or anti-glycemic medication or obesity or any anthropomorphic characteristics of the latter such as weight-to height or waist-hip ratio. Multivariate stepwise backward regression analysis revealed that gender ($\beta = -0.196$, $p = 0.003$), stage of PAD ($\beta = 0.164$, $p = 0.011$), smoking ($\beta = 0.135$, $p = 0.037$), and medication with beta-blockers ($\beta = 0.165$, $p = 0.011$) together explained Vaspin. **Conclusion:** We clearly demonstrate that in our cross-sectional study Vaspin was not related to parameters of obesity. Among characteristics of pre-/diabetes, Vaspin was only associated with fasting insulin. Among all parameters of atherosclerosis such as staging and events, Vaspin only correlated with the rather subjective clinical staging of PAD after Fontaine but not with ankle-brachial index. Thus, we doubt a role of Vaspin in human clinical overt atherosclerosis as well as in manifest obesity of elderly patients.

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Adiposity and diabetic status influences PLA2 isoforms in human abdominal subcutaneous and omental adipose tissue

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Background and aims: Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a member of the phospholipase A2 family of enzymes that hydrolyse phospholipids. It is upregulated in conditions of arterial inflammation and CVD, with macrophages considered the major source. Secreted soluble Lp-PLA2 (sPLA2) is known to directly increase arterial inflammation whilst cytosolic calcium dependent PLA2 (cPLA2) and calcium independent PLA2 (iPLA2) appear to contribute to this inflammation through production of lipid mediators. Limited analysis has however examined conditions of obesity, type 2 diabetes mellitus (T2DM), with previous studies suggesting adiposity correlates with Lp-PLA2 activity and metabolic risk. Therefore, in the present study, we sought to understand the role of the adipocyte through the following: (1) characterising Lp-PLA2 gene expression by microarray and qRT-PCR in human matched paired lean and obese abdominal subcutaneous (Abd Sc) and omental (Abd Om) adipose tissue (AT) (2) determining Lp-PLA2 gene expression in cellular fractions of human Sc and Om AT (3) identifying the protein expression of cPLA2 and iPLA2 in lean and obese matched paired human Abd Sc and omental Abd Om AT (4) evaluating the role of lipids and inflammatory markers on circulating sPLA2 levels in subjects with and without obesity and T2DM.

Materials and methods: Serum was taken from lean, (44.4±6.2yr; BMI: 22.6±2.1kg/m², n=15) overweight (45.4±12.3yr; BMI: 27.1±1.6kg/m², n=20) obese (49.0±9.1yr; BMI: 34.5±3.9kg/m², n=13) and T2DM subjects (53.0±6.13yr; BMI: 43.9±7.0kg/m², n=13). AT (n=21) was taken from the same subjects for the gene and protein analysis by qRT-PCR and western blotting, respectively.

Results: Microarray expression of Lp-PLA2 from paired human Abd Sc and Om AT (lean, n=5; BMI, 23.0±1.2 kg/m²; obese, n=5; BMI, 33.2 ± 3.1 kg/m²) highlighted that Lp-PLA2 expression was increased in obesity and was significantly altered in Abd Sc AT ($p < 0.001$); whilst smaller changes were noted in Om AT. Subsequent qRT-PCR analysis confirmed microarray findings with both Abd Sc and Abd Om AT increased in obesity by 2 and 1.5 fold respectively (Abd Sc Lean: ΔCt 17.79±0.04 Vs Abd Sc Obese: ΔCt 16.75±0.06, $P < 0.001$; Abd Om Lean: ΔCt 18.13±0.71 Vs Abd Om Obese: ΔCt 17.51±0.52; $P < 0.05$) with Lp-PLA2 gene expression higher in Abd Sc than Om AT. Lp-PLA2 gene expression in isolated Sc and Om adipocytes and cultured Sc and Om pre-adipocytes was assessed. cPLA2 protein expression increased with obesity in Abd Sc AT ($p < 0.05$) and depot specific protein expression was measured for iPLA2 across lean, overweight and obese AT samples ($p < 0.05$). Serum Lp-PLA2 significantly correlated with insulin ($r = -0.554$, $p < 0.05$) and leptin ($r = 0.727$, $p < 0.01$) in the obese group, whilst in T2DM subjects Lp-PLA2 inversely correlated with HDL ($r = -0.628$, $p < 0.01$).

Conclusion: Our data highlight that the adipocyte is an active source of Lp-PLA2 which is increased in obesity and altered in an abdominal depot specific manner. Furthermore, in obesity, circulating levels of sPLA2 are influenced by insulin and leptin and inversely correlated with HDL in T2DM. Taken together, these data suggest that in addition to macrophage induced sPLA2

the adipocyte itself may represent a further active site of Lp-PLA2 activity which is altered in an insulin resistant state affecting both lipid metabolism and the inflammatory response.

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Metabolic profile in women with Dunnigan-type partial familial lipodystrophy caused by R482W mutation in the LMNA gene

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Background and aims: Lipotrophic diabetes, also referred to as Dunnigan-Type Partial Familial Lipodystrophy (LPFD), is a rare disease, metabolically characterized by hypertriglyceridemia and insulin resistance. Mutations within the LMNA gene on chromosome 1q21-q23 were recently reported to result in the phenotype of familial partial lipodystrophy. The objective of the present study was to evaluate and compare the metabolic profile of women with and without type 2 diabetes mellitus (T2DM) diagnosed with LPFD.

Materials and methods: A quantitative, descriptive cross-sectional study was conducted on 13 women diagnosed with LPFD followed at the Clinic of Endocrinology and Metabolism - Ribeirão Preto Medical School, Brazil: 08 with and 05 without T2DM, with the determination of anthropometric variables (weight, height, BMI and abdominal circumference). Blood was collected after a 12-h overnight fast for analysis of glucose, glycated hemoglobin (HbA_{1c}), insulin, total cholesterol (TC), triglycerides (TG), HDL cholesterol and uric acid.

Results: Data were analyzed using SAS/STAT software. The Wilcoxon test was used to compare the characteristics of the participants. The data for women with T2DM were: age: 40±12 years, weight: 60.4±3.61 kg, height: 1.60±0.05 m, BMI: 23.6±2.1 kg/m², abdominal circumference: 79±3.5 cm and for those without T2DM (NT2DM), age: 28.4±16.4 years, weight: 57.6±8.09 kg, height: 1.63±0.04 m, BMI: 21.7±1.9 kg/m², abdominal circumference: 73±3.8 cm. There was significant difference between women with T2DM regarding glucose (T2DM: 180.3±79.6 mg/dl vs NT2DM: 78.2±5.81 mg/dl $p < 0.05$), HbA_{1c} (T2DM: 8.8±2.4% vs NT2DM: 6.4±2%; $p < 0.05$), insulin (T2DM: 44.2±29.6 µU/ml vs NT2DM: 27.1±30.1 µU/ml; $p < 0.05$), TG (T2DM: 354.7±207.9 mg/dl vs NT2DM: 207±94.7 mg/dl; $p < 0.05$). There was no significant difference regarding TC (T2DM: 193.7±49 mg/dl vs NT2DM: 209.6±64.6 mg/dl), HDL (T2DM: 37.6±4.1 mg/dl vs NT2DM: 37±6.6 mg/dl) and uric acid (T2DM: 5.1±1.5 mg/dl vs NT2DM: 5.6±1.3 mg/dl).

Conclusion: The present data demonstrate that dyslipemia is an early and prominent feature in the presented lipodystrophic family carrying the R482W mutation within LMNA. Therefore, LMNA encoding lamin A/C may play a role in the genesis of familial combined hyperlipidemia. A better understanding of this rare disease may also have broad clinical implications for the pathological mechanism of common diseases such as dyslipemia and insulin-resistant diabetes mellitus.

Supported by: FAEPA

PS 119 Biomarkers

1276

NT-pro brain natriuretic peptide levels increase during low calorie diet in the obese but return to baseline during weight maintenance unrelated to changes in cardiac function or structure

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Background and aims: Brain natriuretic peptide (BNP) levels are increased in subjects with heart failure due to increased myocyte stretch, and are monitored for diagnosis and prognosis. Paradoxically, obese subjects have shown to have lower levels of BNP compared to lean without relation to heart structure or function. Additionally, a lipolytic action, potentiated during low calorie diet (LCD), has been described. The role for BNP as a biomarker in obese subjects with heart failure has been debated and lower cut-off values are suggested. The aim of this study was to examine the variance in the levels of the BNP N-terminal cleavage equivalent, NT-proBNP, in obese subjects during LCD-induced weight loss and after a period of weight maintenance and to assess its relation to body composition as well as metabolic and echocardiographic measurements.

Materials and methods: 18 obese but otherwise healthy subjects (4 men, 14 women; mean \pm SEM age 38 ± 2.6 years) were recruited. Fasting blood samples were collected and OGTT, body composition measurement as well as echocardiography ($n=12$) were performed before and after a 3 month LCD period (800–880 kcal/day; protein 25–30 E%, carbohydrates 49–55 E%, fat 20–21 E%) and after a 6 month follow up period.

Results: BMI decreased during the LCD period (43.3 ± 1.3 to 35.8 ± 1.8 kg/m², $p=0.0015$) and was maintained during the follow up period (-0.064 kg/m², $p=0.97$). Immediate post weight loss evaluation showed lower levels of blood lipids, lower fasting insulin levels, and lower basal metabolism measures compared to the weight maintenance phase, indicating a more catabolic state. NT-proBNP increased during weight loss (63.4 ± 12.4 to 110.2 ± 26.2 pg/ml, $p=0.033$) but returned to baseline values during weight maintenance (-49.0 pg/ml, $p=0.0047$). Left ventricular mass adjusted for height (LVMI) decreased during weight loss (49.4 ± 3.6 to 36.9 ± 2.6 g/m^{2.7}, $p=0.043$) but increased again slightly during weight maintenance ($+5.0$ g/m^{2.7}, $p=0.043$). All subjects had a preserved systolic function based on left ventricular ejection fraction (LVEF) and this remained stable (60.3 ± 2.2 to 59.2 ± 1.7 % during weight loss, $p=0.39$; $+0.67$ % points during weight maintenance, $p=0.92$). The E/E' index (peak early diastolic blood flow velocity over the mitral annulus [E max]/mean mitral annulus tissue velocity [E' lateral and E' septal]), which relates to the diastolic function, did not change during the study (7.1 ± 0.53 to 6.4 ± 0.59 , $p=0.25$ during weight loss; $+0.53$ during weight maintenance, $p=0.93$).

Conclusion: We show that the increase in BNP during weight loss seems to be associated with the catabolic state during LCD unrelated to the kilograms lost per se, since the levels return during weight maintenance, and unrelated to changes in heart function or structure. An explanation for these observations could be that natriuretic peptides have an important lipolytic role when energy access is decreased. It might be hypothesized that BNP increases in order to ensure access of fatty acids, the most important energy resource for the myocardium, during weight loss. More research on the relation of BNP levels to intake of fat or other sources of energy is needed. Our findings show that not only BMI but also current weight loss should be considered when evaluating BNP levels in obese.

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The relation between eicosapentaenoic acid and factors of atherosclerosis and inflammation in diabetic patients

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Background and aims: Eicosapentaenoic acid (EPA) is an omega-3 fatty acid, which is obtained in the human diet by fish oil. Several reports suggested that EPA prevented atherosclerosis with multiple biological functions depending on lipid or not. Recently, the Japan EPA lipid intervention study reported that long-term treatment with EPA made 22% decreased in the coronary artery

disease (CAD) in impaired glucose patients as compared to 18% decreased in healthy people. It is not clear the mechanisms of EPA to prevent CAD in diabetic patients. The aim of this study is to investigate the relation between plasma EPA level and factors of atherosclerosis and inflammation in diabetic patients without lipid treatment.

Materials and methods: Forty-seven diabetic patients without treatment of anti-hyper lipidemic drugs such as statins and fibrates (males; 47%, age; 58 ± 17 years (mean \pm SD)) were recruited and measured fasting plasma EPA and arachidonic acid (AA) levels. We interviewed number of days eating fish per week, and history of CAD and stroke. Pulse wave velocity (PWV), ankle brachial pressure index (ABI) and intima-media thickness (IMT) were performed as factors of atherosclerosis. The left ventricular (LV) structure and function were measured by echocardiography, and the LV mass index (LVMI) was calculated using Penn's formula. To assessed factors of inflammation, WBC, CRP, h-CRP, and IL-6 were measured.

Results: Average EPA was 44.6 ± 20.8 μ g/ml, AA was 146.2 ± 33.6 μ g/ml, EPA/AA ratio was 0.31 ± 0.14 , and all parameters were normal range. Average number of intake fish was 3.4 ± 2.4 times/week, and was positively correlated with EPA and EPA/AA ($p<0.008$, $p<0.009$, respectively). EPA and EPA/AA were negatively correlated with total number of white blood cell (WBC 5962 ± 1990 / μ l), while CRP, h-CRP, and IL-6 were not correlated significantly. Average HbA1c was 8.9 ± 2.4 %, LDL-C 115 ± 27 mg/dl, HDL-C 49 ± 14 mg/dl, triglyceride 126 ± 64 mg/dl. There was not significantly correlation between EPA and lipids or HbA1c. Moreover, there was no correlation EPA and PWV, ABI, IMT, and LV structure and function.

Conclusion: It suggests EPA may play a role of inflammation system to prevent CAD, independent on lipid and glucose metabolism.

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Organ-specific candidate biomarkers of inflammation found by comparative analyses of the human hepatic and adipose tissue transcriptome and secretome during LPS treatment

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Background and aims: Insulin resistance (IR) is accompanied by chronic low grade systemic inflammation and deregulation of total body energy homeostasis. We induced inflammation in human adipose and human liver tissue *in vitro* in order to mimic inflammation *in vivo* with the aim to identify tissue-specific processes implicated in IR and to find biomarkers indicative for tissue-specific IR.

Methods: Human adipose and liver tissues were cultured in the absence or presence of LPS and DNA Microarray Technology was applied for their transcriptome analysis. Gene Ontology (GO), gene functional analysis, and prediction of genes encoding for secretome were performed using publicly available bioinformatics tools (DAVID, STRING, SecretomeP). The transcriptome data were validated by proteomics analysis of the inflamed adipose tissue secretome using CILAIR technology.

Results: LPS treatment significantly affected 667 and 484 genes in adipose and liver tissues respectively. The GO analysis revealed that during inflammation adipose tissue, compared to liver tissue, had more significantly up-regulated genes, GO terms, and functional clusters related to inflammation and angiogenesis. The secretome prediction led to identification of 399 and 236 genes in adipose and liver tissue respectively. The secretomes of both tissues shared 66 genes and the remaining genes were the differential candidate biomarkers indicative for inflamed adipose or liver tissue. The transcriptome data of the inflamed adipose tissue secretome showed excellent correlation with the proteomics data.

Conclusions: The higher number of altered proinflammatory genes, GO processes, and genes encoding for secretome during inflammation in adipose tissue compared to liver tissue suggests that adipose tissue is the major organ contributing to the development of systemic inflammation observed in IR. The identified tissue specific functional clusters and biomarkers might be used in a strategy for the development of tissue-targeted treatment of IR patients.

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1279

Higher plasma high-mobility group box 1 levels are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study

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Background and aims: High-mobility group box 1 (HMGB1) is a pro-inflammatory cytokine that may contribute to the pathogenesis of vascular complications commonly observed in diabetes. In a cross-sectional study in patients with type 1 diabetes we found serum HMGB1 levels to be positively associated with albuminuria, but not with prevalent cardiovascular disease (CVD). In the present study, we investigated whether plasma HMGB1 levels are associated with incident CVD and all-cause mortality in type 1 diabetes, and the extent to which any such associations could be explained by low-grade inflammation (LGI), endothelial and renal dysfunction, and pulse pressure (PP, a marker of arterial stiffness).

Materials and methods: We prospectively followed 165 individuals with diabetic nephropathy and 168 individuals with persistent normoalbuminuria who were free of CVD at study entry and in whom levels of HMGB1 and other biomarkers were measured at baseline. We used linear and Cox proportional hazards regression analyses adjusted for age, sex, duration of diabetes, case-control status, HbA1c, body mass index, smoking status, total cholesterol, mean arterial pressure, and antihypertensive medication.

Results: Plasma levels of Ln-HMGB1 were not associated with markers of LGI, endothelial and renal dysfunction, and PP. During the course of follow-up [median: 12.3 years (7.8–12.5)], 80 individuals died; 82 suffered a fatal (n=46) and/or nonfatal (n=53) CVD event. Incident fatal and nonfatal CVD and all-cause mortality increased with higher baseline plasma levels of Ln-HMGB1: HR=1.55 (95%CI: 0.94; 2.48) and HR=1.86 (1.18; 2.93) per unit increase in Ln-HMGB1, respectively. Further adjustments for LGI, endothelial and renal dysfunction, and PP did not attenuate these associations.

Conclusion: Plasma HMGB1 levels are positively associated with incident all-cause mortality in type 1 diabetes, independently of conventional cardiovascular risk factors and markers of several potential HMGB1-related pathophysiological mechanisms. To a lesser extent, higher plasma HMGB1 levels are also associated with incident CVD. Thus, HMGB1 may explain, in part, the increased risk of CVD and mortality in these patients. However, the positive association between plasma HMGB1 and incident CVD observed in the present study together with our earlier finding of no association between serum HMGB1 levels and prevalent CVD may suggest that serum levels and plasma levels of HMGB1 do not represent the same pool of HMGB1. Further studies are needed to clarify both the interrelationship between serum and plasma levels of HMGB1, and their associations with CVD.

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Might increased alpha- and beta-defensin levels contribute to the pathogenesis of diabetic complications?

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Background and aims: As defensins are involved in inflammatory and proatherogenic processes, their possible role in the pathogenesis of diabetic micro- and macroangiopathy is also assumed. The aim of this study was to analyse the genetic characteristics and the plasma levels of alpha- and beta-defensins in diabetic patients.

Materials and methods: 98 type 1 and 135 type 2 diabetic patients and 221 controls were tested. The plasma levels of alpha-defensin, the expression of alpha-defensin mRNA and the gene copy numbers of defensin alpha1- alpha3

(DEFA1A3) were measured. Three single nucleotide polymorphisms (SNPs) of the beta-defensin 1 were analyzed. ELISA, real-time PCR, Taq-Man based real-time PCR and Custom TaqMan[®] SNP genotyping method were applied. **Results:** The plasma levels of alpha-defensin were higher in both types of diabetes than in the control group (type 1 vs control: 29030±5650 vs 11940±2960 pg/ml, p<0.001; type 2 vs control: 29800±6010 vs 11940±2960 pg/ml, p<0.001, mean±SEM). The highest concentrations of alpha-defensin were found in diabetic patients with nephropathy (49200±1300 pg/ml vs. 23500±900 pg/ml; with vs without nephropathy; p<0.05), neuropathy (36500±4900 pg/ml vs 25700±3500 pg/ml; with vs without neuropathy; p<0.05) or with cardiovascular complications (45600±1450 pg/ml vs 24500±2500 pg/ml; with vs without cardiovascular complications, p<0.05). The higher alpha-defensin values did not correlate with the DEFA1A3 copy numbers, the mRNA expression or the HbA1c in diabetic patients. Two of the three SNPs of beta-defensin 1 (G20A and G52A) in diabetic patients did not differ from the controls, while the frequency of the GG genotype in the SNP of C-44G allele was lower in both types of diabetes than the controls (2.5% vs 9.5%, p<0.01). This difference was more pronounced in patients with neuropathy or nephropathy compared to controls (1.2% vs 9.5%, p<0.01 and 1.5% vs 9.5%, p<0.01). The CC genotype of C-44G SNP was more frequent among diabetic patients than in the controls (65% vs 50%, p<0.05).

Conclusion: Diabetic patients had increased alpha-defensin levels, the highest values were observed in patients with micro- or macrovascular complications. The presence of certain alleles of beta-defensin 1 seems to be protective against nephropathy or neuropathy in both types of diabetes. These data support a hypothesis that both alpha- and beta-defensins might have an important role in the pathogenesis of diabetic complications.

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Vitamin D deficiency is associated with endothelial dysfunction, in subjects with type 2 diabetes

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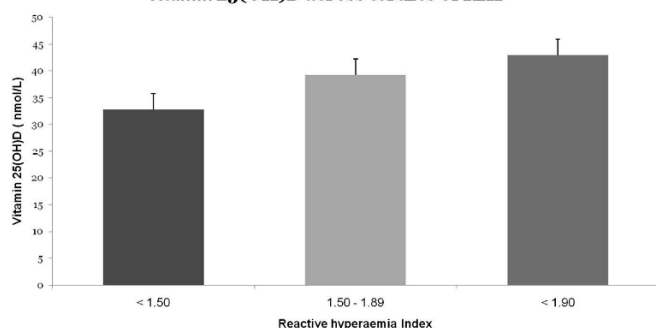
Background and aims: Cardiovascular disease is prevalent in subjects with type 2 diabetes, and both arterial stiffness and endothelial dysfunction may contribute in the pathogenesis. Moreover, epidemiological studies suggest that low levels of vitamin D are associated with both type 2 diabetes and cardiovascular disease. We therefore evaluated possible predictors of aortic stiffness and endothelial function in subjects with type 2 diabetes.

Material and methods: Endothelial function was assessed by measuring the reactive hyperaemia index (RHI) using endothelial pulse amplitude testing (Endo-PAT). Aortic stiffness was estimated as carotid-femoral pulse wave velocity (cfPWV) with applanation tonometry (SphygmoCor) and insulin sensitivity measured with the euglycaemic, hyperinsulinaemic clamp. 25-hydroxy-vitamin D (25(OH)D) was measured using the DiaSorin radioimmunoassay.

Results: Subjects (n=34, 65 % males) were of Nordic (n=24) and South-Indian (n=10) ethnicity and their mean (SD) age was 57.1 (10.2) years, BMI 31.0 (5.1) kg/m², HbA1c 7.6 (1.3)% and diabetes duration 10.3 (6.1) years, 56.7 % had microalbuminuria and all had 25(OH)D <50 nmol/L. Mean (SD) 25(OH)D was 38.4 (8.5) nmol/L, insulin sensitivity, measured as the glucose infusion rate, 734 (436) µmol/m²/min, cfPWV 10.1 (1.9) m/s and RHI 1.77 (0.43). cfPWV correlated positively with age (r=0.46, p=0.008), BMI (r=0.48, p=0.005), systolic blood pressure (0.53, p=0.002) and mean arterial pressure (r=0.43, p=0.01). In multivariate regression analysis, only age (β=0.064, p=0.02) and BMI (β=0.141, p=0.008) were predictors of cfPWV (model R=0.73). Endothelial dysfunction correlated positively with age (r=0.37, p=0.03) and 25(OH)D (r=0.54, p=0.001), and in multivariate regression analysis only 25(OH)D (β=0.019, p=0.03) predicted RHI (model R=0.49). Vitamin 25(OH)D differed significantly across tertiles of RHI (figure 1), p=0.012, with the lowest concentrations in those with the most impaired endothelial function. Insulin sensitivity, HbA1c, diabetes duration and microalbuminuria did not predict cfPWV or endothelial dysfunction.

Conclusion: In subjects with type 2 diabetes, age and BMI predicted aortic stiffness (aPWV) and only 25(OH)D predicted endothelial dysfunction (RHI). Vitamin D seems to be a potential modifier of endothelial function, but not aortic stiffness, in subjects with type 2 diabetes.

Vitamin 25(OH)D across tertiles of RHI



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Plasma procalcitonin and mortality in patients with type 2 diabetes (ZODIAC-27)

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Background and aims: Plasma procalcitonin (PCT) is a known biomarker of bacterial sepsis. More recently, PCT variation at lower plasma levels has been suggested as a new biomarker for chronic low-grade inflammation which is associated with obesity and atherosclerosis. We aimed to investigate whether plasma PCT is related to cardiovascular and all-cause mortality in patients with type 2 diabetes.

Materials and methods: This study is part of the ZODIAC study, a prospective observational study amongst patients with type 2 diabetes treated in primary care in the Netherlands. A total of 1192 patients, who started to participate between 1998 and 2002 were included in the study. Blood was drawn for future research. In 2009 life status and cause of death were assessed. The relationship between PCT and mortality was investigated using Cox proportional hazard analyses. The analyses were performed with PCT categorised into quintiles and also as a continuous variable. Three models were chosen: a crude model, a model in which was adjusted for age and gender and finally a model in which we additionally adjusted for BMI, serum creatinine, smoking, diabetes duration, systolic blood pressure, cholesterol HDL ratio, history of macrovascular complications and albuminuria. PCT and serum creatinine were logarithmically transformed because of skewed distribution of the data. All analyses were performed after excluding PCT values above 0.1 ng/ml (n=8).

Results: Mean age of the study cohort was 66.7 ± 11.6 years, and 521 (43.7%) patients were male. Median [interquartile range] PCT level was 0.020 [0.014-0.022] ng/mL. Further baseline data include a median HbA1c of 53 [45-65] mmol/mol, diabetes duration of 4 [2-9] years, estimated creatinine clearance of 72 [57-91] mL/min and urinary albumin creatinine ratio of 1.92 [0.93-6.58] mg/mmol. After median follow-up of 9.8 years, 348 (29%) out of 1191 patients had died. The unadjusted Hazard Ratio (HR) for all-cause mortality was 1.88 (95% CI 1.33-2.65) for patients in the highest quintile (>0.024 ng/mL) of PCT compared to the lowest quintile. In the second and third model these HRs were 1.39 (95% CI 0.98-1.96) and 1.16 (95% CI 0.80-1.68), respectively. For PCT on a continuous scale, the unadjusted HR for logPCT was 1.74 (95% CI 1.34-2.27). In the second and third model these HRs were 1.43 (95% CI 1.09-1.88) and 1.22 (95% CI 0.91-1.63), respectively. For CV mortality, the HRs for logPCT were 1.62 (95% CI 1.08-2.43), 1.34 (95% CI 0.88-2.05) and 0.79 (95% CI 0.50-1.25) in model 1, 2 and 3, respectively. The HRs for the highest PCT quintile compared to the lowest were 1.89 (95% CI 1.12-3.19), 1.42 (95% CI 0.84-2.40) and 0.83 (95% CI 0.47-1.47) in model 1, 2 and 3, respectively.

Conclusion: Elevated plasma PCT levels, in the normal range, were associated with mortality. However, this relationship is to a large extent confounded by well-established risk factors in patients with type 2 diabetes. Based on the confidence intervals, a small independent relation between PCT and mortality cannot be fully excluded.

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Osteoprotegerin and coronary artery disease in type 2 diabetic patients with microalbuminuria

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Background and aims: Plasma osteoprotegerin (P-OPG) is a strong and independent predictor of cardiovascular disease (CVD) in diabetic and other populations. OPG is a bone-related glycopeptide produced by vascular smooth muscle cells and increased P-OPG may reflect arterial damage. We investigated the correlation between P-OPG and coronary artery disease (CAD) in asymptomatic type 2 diabetic patients with microalbuminuria.

Methods: P-OPG was measured in 200 asymptomatic type 2 diabetic patients without previous cardiac disease. Patients with P-NT-proBNP >45.2 ng/L and/or coronary calcium score (CCS) ≥ 400 were stratified as high risk of CAD (n=133), and all other patients as low risk patients (n=67). High risk patients were examined by myocardial perfusion imaging (MPI; n=109), and/or CT-angiography (n=20), and/or coronary angiography (CAG; n=86). Significant CAD was defined by presence of significant myocardial perfusion defects at MPI or $>70\%$ coronary artery stenosis at CAG.

Results: Significant CAD was demonstrated in 70 of the 200 patients and of these 23 patients had $>70\%$ coronary artery stenosis at CAG. P-OPG was higher in patients with significant CAD compared to patients without CAD (n=130). Increased P-OPG was an independent predictor of significant CAD (adjusted odds ratio [CI] 3.51 [1.39-8.86] and 3.54 [1.36-9.21] for second and third tertile vs. first tertile P-OPG, respectively) and remained so after adjustments for NT-proBNP and CCS. The area under the receiver-operating curve in a model for prediction of significant CAD changed from 84% to 87%, when P-OPG was added to the model. High P-OPG was also associated with presence of $>70\%$ coronary artery stenosis (adjusted odds ratio 14.20 [1.35-148.92] for third vs. first tertile P-OPG), and 91% of patients with low (first tertile) P-OPG did not have $>70\%$ coronary artery stenosis.

Conclusions: Elevated P-OPG is an independent predictor of the presence of CAD in asymptomatic type 2 diabetic patients with microalbuminuria.

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1284

Chemerin is independently associated with albuminuria in type 2 diabetic patients

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Background and aims: Chemerin is a recently discovered metabolic regulator hormone. Moreover, chemerin has been suggested to be linked to obesity-induced insulin resistance and type 2 diabetes. Diabetic nephropathy is a serious microvascular complication of diabetes mellitus. The decrease in renal function of diabetic nephropathy is characterized by elevated urinary albumin excretion. Recently, serum chemerin levels were found to be significantly associated with renal dysfunction in type 2 diabetic patients. However, the association of plasma chemerin levels with albuminuria is unknown yet. The aim of the study was to determine whether serum chemerin levels are associated with albuminuria in type 2 diabetic patients.

Materials and methods: One hundred two patients with type 2 diabetes were enrolled. These patients were newly diagnosed with type 2 diabetes and were drug naïve. Blood pressure, body mass index (BMI), waist circumference (WC) and serum concentration of A1C, glucose, insulin, lipid profile, hsCRP, and chemerin were measured. Insulin resistance was estimated by the short insulin tolerance test. Visceral fat thickness was determined by ultrasonography. Urine albumin excretion (UAE) was assessed as albumin:creatinine ratio in early-morning urine samples. The glomerular

filtration rate (GFR) was calculated using the Cockcroft and Gault formula. Serum levels of chemerin were measured by enzyme-linked immunosorbent assay.

Results: The clinical characteristics of the control group and the diabetic group are presented in Table 1. No differences were found in levels of chemerin between female and male. Serum chemerin level was significantly correlated with BMI ($r=0.343$, $p<0.001$), WC ($r=0.36$, $p=0.001$), VFT ($r=0.390$, $p<0.001$), insulin ($r=0.452$, $p=0.000$), triglyceride ($r=0.295$, $p=0.003$), HDL-cholesterol ($r=-0.318$, $p=0.002$), hsCRP (log-transformed, $r=0.263$, $p=0.037$) and UAE (log-transformed, $r=0.275$, $p=0.007$). Moreover, after adjustment for glucose, blood pressure, multiple regression analysis showed that serum chemerin was significantly associated with urine albumin excretion ($\beta=0.256$, $p=0.011$).

Conclusion: Serum chemerin is another important independent factor associated with albuminuria in type 2 diabetic patients.

Table 1. Clinical and biochemical characteristics of type 2 diabetic patients

	Female	Male	P-value
N	39	63	
Age (years)	51.4 ± 11.3	46.4 ± 9.1	0.017
BMI (kg/m ²)	25.4 ± 3.7	25.8 ± 3.4	0.554
WC (cm)	87.8 ± 8.9	92.887 ± 9.245	0.008
VFT (mm)	38.6 ± 11.8	46.2 ± 13.4	0.005
SBP (mmHg)	128.0 ± 15.3	127.0 ± 16.8	0.760
DBP (mmHg)	77.1 ± 10.6	80.2 ± 9.9	0.141
A1C (%)	7.6 ± 1.6	7.9 ± 1.9	0.403
FBS (mg/dl)	137.6 ± 42.9	153.1 ± 55.9	0.145
PP2G (mg/dl)	182.8 ± 66.2	212.8 ± 90.4	0.086
Kitt (%/min)	2.5 ± 1.2	2.3 ± 1.1	0.614
Insulin (μU/ml)	8.3 ± 4.7	7.7 ± 5.2	0.638
C-peptide (ng/ml)	2.2 ± 0.9	2.6 ± 1.4	0.152
hsCRP (mg/l)	1.7 ± 1.6	1.5 ± 1.8	0.813
BUN (mg/dl)	14.6 ± 4.6	15.4 ± 4.5	0.408
Cr (mg/dl)	0.7 ± 0.1	0.9 ± 0.1	0.000
GFR (ml/min/1.73m ²)	103.0 ± 23.6	103.5 ± 23.5	0.925
TC (mg/dl)	177.5 ± 33.8	185.4 ± 37.5	0.293
TG (mg/dl)	130.6 ± 59.8	158.1 ± 90.1	0.098
HDL-C (mg/dl)	49.5 ± 11.6	45.5 ± 11.7	0.124
LDL-C (mg/dl)	103.0 ± 31.6	108.0 ± 32.4	0.453
Chemerin (ng/ml)	45.0 ± 19.8	40.0 ± 15.3	0.157
UAE (mg albumin/g Cr)	19.4 ± 27.5	15.2 ± 25.5	0.453

Data are mean ± SD. BMI, body mass index; WC, waist circumference; VFT, visceral fat thickness; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; PP2G, postprandial 2 hr glucose; hsCRP, high sensitive C-reactive protein; BUN, blood urea nitrogen; Cr, creatinine; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UAE, urine albumin excretion

Clinical Trial Registration Number: 2009-S051

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PS 120 Novel complications

1285

Neuronal and glial changes induced by type 1 diabetes in the rat hippocampus

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Background and aims: Long-term diabetes can result in a variety of subtle cerebral disorders with manifestations demonstrated at structural, neurochemical, electrophysiological and neurobehavioural level. Cognitive and memory impairments associated with diabetes result from modification of hippocampal structure and function, including changes in neuronal and glial cells. The aim of this work was to further evaluate and identify changes in hippocampal neuronal and glial cells induced by diabetes at different time points along the early stages of the disease (two, four and eight weeks).

Materials and methods: Diabetes was induced in male Wistar rats with a single intraperitoneal injection of streptozotocin. Neurodegeneration and apoptosis was evaluated by cresyl violet staining and detection of caspase-3. Neuronal changes were assessed by evaluating the immunoreactivity of microtubule associated protein-2 (MAP-2) and synaptophysin, as well as the protein content of Tuj-1, calbindin D28k and tau. Astrocyte reactivity was analyzed by measuring the content and immunoreactivity of glial fibrillary acidic protein (GFAP). Changes in microglial cells were evaluated with CD11b and ED1 markers.

Results: No signs of neurodegeneration or caspase-3 activation were detected in the hippocampus of diabetic animals. However, a decrease in the protein levels of all neuronal markers occurred after eight weeks of diabetes. Regarding MAP-2 immunoreactivity, there was a decrease in dentate gyrus at all time points of the study, and after eight weeks a decrease occurred in all hippocampal subregions (CA1, CA3 and dentate gyrus). Synaptophysin immunoreactivity increased in CA3 subregion at all time points. No changes were detected in astrocyte reactivity in diabetic animals. However, the number of activated microglia increased in dentate gyrus and CA3 subregion at eight weeks of diabetes.

Discussion: These observations indicate that diabetes does not induce neuronal degeneration in the hippocampus, at least during the early stages of the disease. However, neuronal changes occur, mainly after longer periods of diabetes (eight weeks), and CA3 subregion appears to be the most affected region. Moreover, astrocytes did not become reactive with diabetes, at least for the time points studied, but microglial cells became activated, suggesting the existence of a pro-inflammatory response. Altogether, these changes might affect cognitive and memory performance.

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1286

Cerebrospinal fluid Alzheimer's disease markers in adult type 1 diabetes patients: associations with microangiopathy and APOE ε4

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Background and aims: Type 1 diabetes (T1DM), especially in the presence of peripheral microangiopathy, is associated with cognitive decrements and cerebral structural changes. Cognitive deficits are mainly found in domains concerning information processing speed and structural brain loss has been observed in grey matter. Furthermore, diabetes in general has been associated with a 2-fold higher risk of dementia in later life, including vascular dementia and Alzheimer's Disease (AD), possibly mediated by apolipoprotein E (APOE) ε4 genotype. Cerebrospinal fluid (CSF) amyloid-β1-42 (Aβ42), total tau and tau phosphorylated at threonine 181 (p-tau-181) are usually changed in dementia. Amyloid deposition is found extracellular and can impair neu-

ronal signaling, leading to cell death. Hyperphosphorylated tau tangles are found intracellular and are a marker of neuronal death. For example, in AD, A β 42 concentrations are decreased whereas tau and ptau-181 are increased. These changes are already present during the early phase of AD. Presently, it is unknown whether these biomarkers are altered in T1DM patients and whether these changes are related to the presence of microangiopathy and APOE ϵ 4.

Materials and methods: Ten patients with (mean age 48.5 years; mean HbA1c 7.5%; mean disease duration 35.6 years), 28 without peripheral microangiopathy (mean age 40.5 years; mean HbA1c 7.8%; mean disease duration 22.8 years) and 15 controls (mean age 39.9 years; mean HbA1c 5.4%), on T1DM versus control level matched for age, gender, BMI and IQ, were included in this study. They underwent a lumbar puncture at L3/L4 or L4/L5 for CSF collection and blood sampling for APOE genotyping. To enhance power we analysed T1DM patients as whole group compared to controls.

Results: Patients with peripheral microangiopathy were oldest and had most depressive symptoms, compared to the other groups. All 3 CSF biomarkers for AD were not changed in T1DM patients compared to controls, although ptau-181 tended to be increased ($P=0.1$). In T1DM patients with APOE ϵ 4 genotype, A β 42 concentrations were lower compared to those without the APOE ϵ 4 genotype ($P=0.033$), which was not observed in controls. Microangiopathy presence did not alter CSF biomarker concentrations.

Conclusion: This sample of T1DM patients with a mean age of 42 years did not show a AD-like profile of decreased CSF A β 42 and increased total tau and ptau-181 levels. However, in T1DM patients with APOE ϵ 4, a significant decrease in A β 42 concentration was observed. In these patients, amyloid deposition in increased compared to their non-APOE ϵ 4 counterparts, which may result in impaired neuronal signaling and cell death. Whether CSF biomarker concentrations change over time and what their predictive value for cognitive functions is, has to be further detailed.

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1287

Cognition in type 1 diabetes mellitus

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Background and aims: Individuals with diabetes are approximately 1.5-fold more likely to experience cognitive decline than individuals without diabetes. Several neuropsychological tests are used to assess multiple cognitive domains. The Stroop Neuropsychological Screening Test and Wisconsin Card Sorting Test (WCST) are widely used in clinical practice and research to examine selective attention, cognitive flexibility, processing speed, the ability to learn concepts, and used as a tool in the evaluation of executive functions. Our aims were to investigate differences in cognitive function between patients with type 1 diabetes mellitus (T1DM) and healthy controls (C), and evaluate the association between cognitive dysfunction and glycemic control in patients.

Materials and methods: 50 T1DM and 50 C were screened for cognitive function with the computerized version of the Stroop test and the WCST. Depression was evaluated with the Hospital Anxiety and Depression Scale. Interview questionnaires surveyed the detailed anamnesis and medication. Anthropometry and metabolic parameters were measured. The computerized version of the original Stroop test recorded congruent and incongruent trials reaction time and calculated the Stroop effect reaction time (SERT). The WCST has shown total number of errors, number of perseverative errors to describe mental flexibility.

Results: The Stroop test showed that the SERT (DMSERT:112.8 \pm 74.16ms), the congruent RT (DMCRT:828.69 \pm 99.3ms) and the incongruent RT (DMICRT:941.58 \pm 127.37ms) of the T1DM group were significantly ($p<0.01$) longer than the C group SERT (CSERT:64 \pm 53.4ms), congruent RT (CCRT:661.44 \pm 85.7ms), and incongruent RT (CICRT:725.38 \pm 85.7ms). A statistically significant association was observed between the HbA1C (7.76 \pm 1.75%) levels and the score on Stroop effect ($p=0.028$). In WCST the rate of correct answers (80.25 \pm 7.55) was negative significantly ($p=0.022$) correlated with duration of T1DM. The two groups were age-adjusted (Cage: 32.18 \pm 11.75yr; T1DMage:35.62 \pm 11.65yr).

Conclusion: T1DM is associated with cognitive deficit, which can be related to many different regions of the brain. Our results indicate that T1DM show impaired executive functions and information-processing speed. Chronic

poorly controlled carbohydrate metabolism might have a role in the development of selective attention deficit. Optimal diabetes care can improve the motor speed, attention and executive function.

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Poor glycaemic control is associated with cognitive dysfunction in older adults with type 2 diabetes

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Background and aims: Type 2 Diabetes (DM2) is associated with a high risk of Cognitive Dysfunction (CD) and dementia. The relationship between glycemic control and cognitive function remains unclear. We analyze the association between glycemic control (A1c) and cognitive dysfunction in older adults with DM 2 without a diagnosis of dementia.

Materials and methods: Patients with DM ≥ 65 years old were studied. The study was carried out by 17 Argentinean doctors specialized in Diabetes from the states of Buenos Aires, Salta and Buenos Aires City (Argentina). The evaluation included the following items: duration of diabetes, treatment and complications of diabetes type 2, level of education, income, habits, anthropometric measures and laboratory tests. The metabolic control was measured by A1c value. Cognitive function was evaluated by the test Mini Mental State Examination (MMSE), (normal 28-30, cognitive dysfunction ≤ 27 points) and depression by the Yessavage depression scale (considered pathologic > 9 points). Statistical Analysis: Chi2, Wilcoxon Test and Multiple Logistic Regression. Software Intercooled STATA 9.0.

Results: 427 patients were studied, female 55.2%, age 71.83 \pm 5.58 years, duration of diabetes 11.78 \pm 9.17 years, glycemia 128 \pm 42.19 mg/dl, A1c 7.15 \pm 2.73 %, BMI 29.71 \pm 5.65 Kg/m2. CD was present in 225 patients (52.7%), and Depression in 79 (18.5%). Education level ≤ 7 years 178 (41.7%), monthly income ≤ 300 euros 116 (27.2%), Alcohol consumption 208 (48.7%), smoking 30 (7.0%), obesity 167 (39.1%) and living alone 63 (14.8%) of the patients. Treatment: 24 patients (5.6%) were without pharmacological treatment, 271 patients (63.5%) with anti-diabetic oral medication and 132 (30.9%) with insulin alone or combined with oral medication. History of hypoglycemia was present in 82 patients (19.2%), dyslipidemia in 370 (86.7%), Hypertension in 376 (88.1%), peripheral arterial disease in 39 (9.1%), coronary disease in 87 (20.4%), carotid disease in 56 (13.1%). The Hb A1c > 7 was associated with CD: rough OR=1.56; CI 95%=1.05-2.33; $p=0.02$. When A1c was adjusted by age, sex, income, education, obesity, physical activity, depression, hypertension, tobacco, alcohol, work, living alone and dyslipidemia, the A1c showed an adjusted OR=1.67; CI95%=1.01-2.76; $p=0.04$. Forward Stepwise Logistic regression was used to identify the predictors for CD. The protective variables were: living alone, (OR: 0.50; CI: 0.28-0.94; $p < 0.03$) and obesity (OR: 0.62; CI:0.40-0.96; $p < 0.03$). The following variables were identified as risk factors for cognitive dysfunction: A1c (OR= 0.52; CI:0.28-0.94; $p<0.02$), depression (OR= 1.80; CI: 1.03-3.15; $p<0.03$), Low income (OR = 2.37-4.03; $p < 0.002$) and less education level (OR= 3.71; CI:2.35-5.86; $p < 0.0001$).

Conclusion: Cognitive dysfunction was found to be a common occurrence in a sample of older diabetic individuals. In this population, the A1c > 7 was associated with CD. Poor glycemic control, low education level, lower income and depression were also identified as risk factors for CD. These findings need to be validated in prospective studies.

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1289

Obesity of the bone: the relationships between body mass, adiposity and bone density in children: a 7-year longitudinal study

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Background and aims: Osteoporosis and obesity share a common progenitor cell, and osteoporosis has been dubbed 'obesity of the bone'. Indeed, osteoporosis is a well-recognised co-morbidity of diabetes, itself largely attributable to obesity. Peak bone mass is an important determinant of osteoporosis, but there is little information on the impact of body composition and metabolic health on bone growth in children.

Materials and methods: The relationship between fatness and bone mineral density (BMD) was investigated annually in 307 healthy children from 5–12y in a prospective cohort study. Fatness was measured by serum leptin (ng/ml) and BMD (g/cm^2) by dual-energy x-ray absorptiometry (DEXA). Leptin was chosen as a measure of fatness for three reasons - it derives exclusively from adipocytes, its concentration is independent of fat-free mass, and it correlates closely with adiposity. BMI (m/kg^2) was deduced from duplicate measures of height and weight and converted to BMI sds, a standardised measure of weight excess. The association between BMD and leptin for each gender and year of growth was analysed using covariance-pattern modelling, controlling for age-related growth and fat-free mass.

Results: BMD ($0.02 \text{ g}/\text{cm}^2$ per year), BMI SDS (0.14 units/year) and serum leptin ($1.5 \text{ ng}/\text{ml}$ per year) all rose annually throughout. There was a clear positive association ($p < 0.001$) between BMD and leptin in both genders from 5y to 8y, with BMD rising on average by $0.009 \text{ g}/\text{cm}^2$ in boys and $0.006 \text{ g}/\text{cm}^2$ in girls, for every $10 \text{ ng}/\text{ml}$ increase of leptin. However between the ages of 8y and 9y the direction of association changes to a negative trend with BMD decreasing on average by $0.002 \text{ g}/\text{cm}^2$ in boys and $0.001 \text{ g}/\text{cm}^2$ in girls for every $10 \text{ ng}/\text{ml}$ increase of leptin.

Conclusions: Diabetes is associated with loss of bone, believed to result from fatty infiltration of the medulla. Although BMD and leptin as BMI sds (excess body fat) continued to rise with age, the initial positive relationship between BMD and leptin is inverted between the ages of 8y and 9y. This inversion may reflect the earliest stages of 'obesity of the bone'.

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1290

Quantitative ultrasound and vertebral fractures in patients with type 2 diabetes

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Background and aims: Patients with type 2 diabetes (T2DM) are known to have increased risks of femoral neck and vertebral fractures, although their bone mineral density (BMD) is normal or even slightly increased compared to non-DM controls. This observation suggests that bone fragility not reflected by BMD, possibly deterioration of bone quality, may participate in their fracture risks. Quantitative ultrasound (QUS), not like BMD, could possibly evaluate bone quality, especially microarchitecture, and therefore might be useful for assessing fracture risk in T2DM.

Materials and methods: To test this hypothesis, we measured calcaneal QUS as well as BMD at the lumbar spine, femoral neck, and 1/3 radius in 96 women (mean age, 66.6 years old) and 99 men (64.7 years old) with T2DM, and examined their associations with prevalent vertebral fractures (VFs). Calcaneal QUS was performed by CM-200, and speed of sound (SOS) values were obtained. BMD was measured by QDR4500.

Results: In T2DM patients, VFs were found in 33 and 45 subjects in women and men, respectively. When compared between subjects with and without VFs, there were no significant differences in values of SOS or BMD at any site between the groups in either gender. The distribution of SOS as a function of age showed that those with VFs were scattered widely and there were no SOS thresholds for VFs in either gender. Logistic regression analysis adjusted for age and BMI showed that either SOS or BMD was not significantly associated with the presence of VFs in either gender.

Conclusion: These results show that QUS as well as BMD are unable to discriminate T2DM patients with prevalent VFs from those without VFs. It seems necessary to seek other imaging modalities or biochemical markers evaluating bone fragility and fracture risk in T2DM.

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The correlation between HbA_{1c} and risk of femoral fractures in elderly patients

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Background and aims: Elderly patients with type 2 diabetes mellitus (T2DM) are at greater risk of falls as a result of chronic hyperglycaemic complications, such as retinopathy and neuropathy, or secondary to treatment-induced hypoglycaemia. Previous studies have shown that tight glycaemic control ($\text{HbA}_{1c} < 7.0\%$) is closely related to an increased risk of falls. The

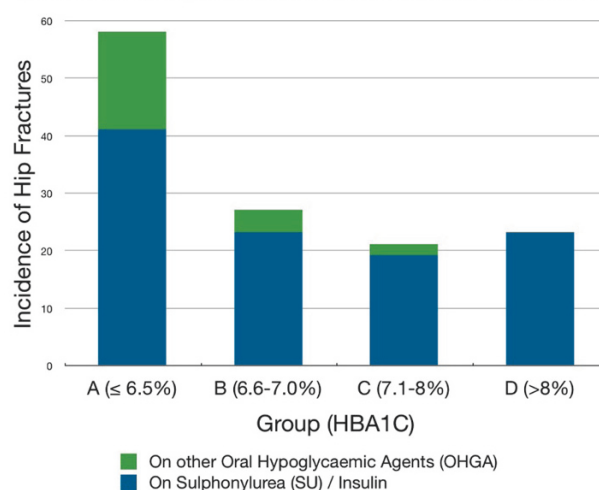
present study sought to evaluate the relationship between glycaemic control and diabetes therapy with the incidence of hip fractures in a tertiary hospital in Singapore.

Materials and methods: All patients with T2DM who were admitted in a 12 month period from Dec 2009 to Dec 2010 inclusive for a low energy hip fracture were included in the analysis. Patients on diet control or newly diagnosed with diabetes on admission were excluded from the study. Clinical details and data from the study were obtained from the hospital laboratory database and discernment of medical notes.

Results: There were a total of 129 patients (88 female, 41 male, mean age 77.1 ± 9.4) included in the study. Median HbA_{1c} was 6.6% (Inter-Quartile Range $6.0\%-7.4\%$). The patients were divided into 4 groups, Group A ($\text{HbA}_{1c} < 6.5\%$), Group B ($\text{HbA}_{1c} 6.5-7.0\%$), Group C ($\text{HbA}_{1c} 7.1-8.0\%$) and Group D ($\text{HbA}_{1c} > 8\%$). The results of the study are summarised in Graph 1. There were significantly more patients with hip fractures in Group A (45.0%) compared with Group B (20.9%), Group C (16.3%) and Group D (17.8%). The patients in Group A were also older (mean age 79.4 ± 8.4) compared to Group B (77.2 ± 9.7 , $p=0.30$), Group C (75.4 ± 8.9 , $p=0.07$), and significantly older than Group D (72.8 ± 10.6 , $p=0.004$) using independent t test. Crucially, a high proportion of these patients in Group A were also receiving sulphonylureas (SU) or insulin therapy (41 of 58, 70.7%), 22 on SU monotherapy, 18 on SU in combination with metformin or acarbose, and 1 on SU with insulin.

Conclusion: Sulphonylureas are associated with an increased risk of hypoglycaemia, particularly in the elderly. Furthermore, low HbA_{1c} values have been shown to increase the risk of falls and hypoglycaemic attacks in this population. While glycaemic targets are set at $< 7\%$, or $< 6.5\%$ for most, the American Geriatrics Society has suggested a less stringent HbA_{1c} therapeutic target of 8% for frail elderly patients. Despite this, the present study shows that nearly half of elderly patients admitted with hip fractures had very tight glycaemic control ($\text{HbA}_{1c} < 6.5\%$). Hip fractures are associated with a high mortality rate in the subsequent years. Hence, prevention of this is of paramount importance. 82.2% of patients who sustained a hip fracture in the present study were on sulphonylureas and / or insulin therapy, of which nearly half had HbA_{1c} values $< 6.5\%$ (38.7%). Although a relationship between hip fractures and the risk of hypoglycaemia in this case can only be inferred, it is clear that there is a need for less stringent glycaemic control in the elderly. Primary physicians need to tailor diabetes therapy based on the side effects profile of intended drugs and to individualised treatment targets in each patient.

Incidence of Hip Fractures in DM Patients on medications



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The impact of modifiable factors on hearing function in diabetic subjects

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Background and aims: Sense of hearing is a very important tool of social communication. Hearing impairment prevalence in diabetic subjects is roughly doubled in comparison with a general population. The aim of our

interdisciplinary study was to assess whether some modifiable factors, like HbA_{1c}, blood pressure, serum lipids and BMI have an impact on auditory organ function in the relatively young diabetic subjects.

Materials and methods: 58 patients, 31 with type 1 diabetes and 27 with type 2 diabetes, aged below 45 years, with a diabetes duration < 10 years, and without clinically overt hearing impairment or diabetic neuropathy, were included. After vital signs and blood samples for laboratory evaluations were obtained, patients were referred to the audiological lab, where a pure-tone audiometry and transiently-evoked otoacoustic emissions (TEOAE) were evaluated. Hearing threshold ≥ 25 dB in at least 1 frequency was recognized as hearing impairment. TEOAE amplitude < 6 dB was considered as an absence of otoacoustic emissions.

Results: 38 patients had a normal hearing. The hearing impairment was found in 20 subjects (34.5 %). These patients had a significantly lower HDL-cholesterol level (44.2 ± 13.4 mg/dl vs. 57.6 ± 13.1 mg/dl, $p < 0.001$) and a higher BMI (29.6 ± 6.3 kg/m² vs. 25.5 ± 6.4 kg/m², $p = 0.011$) in comparison with normal-hearing subjects (Figure). A trend towards higher triglycerides was also observed (132.4 ± 79.5 mg/dl vs. 93.6 ± 59.8 mg/dl, $p = 0.059$). No significant differences between the 2 groups regarding HbA_{1c}, blood pressure and LDL-cholesterol were found. Normal TEOAE amplitude was found in 42 subjects. Absence of otoacoustic emissions was diagnosed in 16 subjects (27.6 %). Also these patients had a lower HDL-cholesterol level (45.4 ± 16.6 mg/dl vs. 55.2 ± 13.2 mg/dl, $p = 0.018$) (Figure). No significant differences between the 2 groups regarding other variables were demonstrated. In the linear correlations analyses, a significant negative relationship between hearing threshold and HDL-cholesterol (at frequencies from 1000 Hz to 12,000 Hz), as well as positive correlation between hearing threshold and triglycerides (at frequencies from 1000 Hz to 4000 Hz), BMI (from 2000 Hz to 12,000 Hz) and systolic blood pressure (SBP) (at 2000 Hz and 3000 Hz) were found. Also significant positive correlation between TEOAE amplitude and HDL-cholesterol ($r = 0.333$, $p = 0.014$), and a negative correlation between TEOAE amplitude and triglycerides ($r = -0.277$, $p = 0.043$) were demonstrated.

Conclusion: Hearing impairment is frequent in diabetic subjects. Our study revealed that several modifiable factors like HDL-cholesterol, triglycerides, SBP and BMI may affect hearing function in this population. If these results were confirmed in further studies, a vast area of possible therapeutic interventions to preserve hearing in diabetic patients would open.

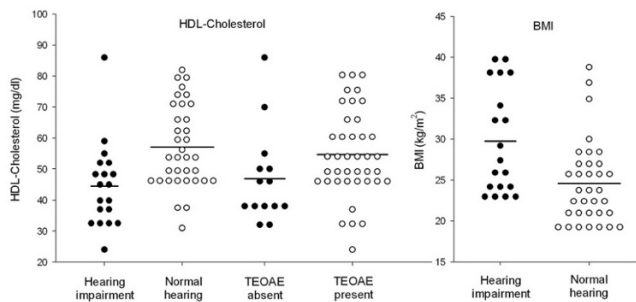


Figure. Histogram plot of HDL-cholesterol level and BMI value in patients with hearing impairment and normal hearing; and in subjects with absent and present otoacoustic emissions.

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Screening on a wide scale and profile of the sleep apnoea syndrome in type 2 diabetes patients

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Background and aims: An effective screening of the sleep apnoea syndrome (SAS) in particular in type 2 diabetes patients (T2D) is impaired by the lack of specificity of the clinical manifestations and the fact that it is not easy to perform a polysomnography on a wide scale. Does the use of pulse oximetry ease the SAS wide scale screening?

Materials and methods: A pulse oximetry has been performed in 586 T2D overweight (BMI ≥ 27 kg/m²) patients disconnected from an emergency hospitalization or an obvious pulmonary pathology. Three criteria suggesting a possible SAS were selected: SpO₂ percentage < 90% (at least ≥ 10 %), Oxygen Desaturation Index (ODI) ≥ 10 , time spent at SpO₂ 30 or > 10 10.

Results: 404 (69%) patients show 0 or 1 criterion, 82 (14%) show 2 criteria and 100 (17%) show 3 criteria. Among those last 182 patients (31%) who show at least 2 criteria, 150 could benefit from a polysomnography. SAS diagnosis was confirmed for 116 patients (20%). 40 patients among the 64

showing 2 criteria (62,5%) and 76 patients among the 86 showing 3 criteria (88%) actually suffered from SAS. The analysis of diagnosed SAS profile concludes with an average age of 65.4 yrs, a sex ratio F/M of 1.09, a BMI of 36 kg/m², 27.5% suffer from a severe coronarite. Among the 150 patients, SAS frequency according to the BMI (kg/m²) is: BMI > 27 30 35 40 : 73% + 8 alveolar hypoventilation syndromes (diagnosis of 95% of obvious respiratory troubles). Regarding the oximetry analysis on these SAS patients, the average SpO₂ < 90% is 36.2%; the average ODI is 23, the average time spent under hypoxia (SpO₂ < 90%) is 161 mn, the average SpO₂ is 86%.

Conclusion: The pulse oximetry offers an efficient wide scale screening, realistic and easy to implement whether externally, especially effective if 3 criteria are present. Taking into account SAS frequency (20%) in overweight T2D patients, its well-known cardiac consequences (27% of severe coronary diseases in this series), the importance of hypoxia, this screening technique appears to us as enough relevant to be generalized.

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Effect of initiation of continuous positive airway pressure therapy on cardiovascular risk factors in patients with type 2 diabetes and obstructive sleep apnoea

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Background and aims: Diabetes and obstructive sleep apnoea (OSA) are important co-morbid conditions. In people with diabetes, the prevalence of OSA may be as high as 58%. Continuous Positive Airway Pressure (CPAP) is a highly effective treatment for OSA but there are limited data on the effect of CPAP therapy on OSA in type 2 patients. It is possible that CPAP could improve metabolic control, blood pressure and diabetes-associated complications such as sexual dysfunction in males. We therefore conducted a before-and-after study to determine whether CPAP improves glycaemic control and other cardiovascular risk factors in type 2 patients with confirmed OSA.

Materials and methods: Between April 2009 and March 2010, 59 type 2 diabetes patients from the longitudinal observational Fremantle Diabetes Study considered at high risk for OSA after an overnight home-based sleep study using an ApneaLink had confirmation of the diagnosis by laboratory polysomnography and then consented to participation in a 3-month CPAP intervention study. A detailed medical history, physical examination and biochemical testing were undertaken immediately before, and 1 and 3 months after CPAP commenced. The males completed two sexual dysfunction questionnaires, the sexual health inventory for men and the ADAM questionnaire, on each occasion. Repeated measures statistical analysis was used to compare variables of interest at the three time points.

Results: Forty-four (75%) of the original cohort completed the study. They had a mean \pm SD age of 66.1 ± 8.8 years, 61.4% were male their median [IQR] diabetes duration was 10.1 [3.8–15.3] years, and they had an apnoea hypopnoea index (AHI) of 38 [27–58] and Epworth Sleepiness Scale (ESS) of 9 [5–12] at entry. There were no significant differences between the 44 completers and 15 non-completers in age, sex, diabetes duration, AHI or ESS ($P \geq 0.29$). Blood pressure improved significantly over 3 months of CPAP therapy from $149 \pm 23/80 \pm 12$ mmHg at entry to $140 \pm 18/73 \pm 13$ mmHg (trend $P = 0.038/0.013$). The change in systolic blood pressure (Δ SBP) was -8 (-18 to 1) mmHg, with significant improvement between 1 month and 3 months (Δ SBP -8 (-14 to -1) mmHg, $P = 0.012$). Most improvement in diastolic blood pressure also occurred between months 1 and 3 (Δ DBP -8 (-15 to -1) mmHg, $P = 0.015$). Pulse rate declined significantly within the first month (Δ pulse rate -6 (-10 to -2) beats/minute, $P = 0.001$). Glycaemic control, serum lipids and urinary albumin:creatinine ratio (ACR) did not improve over the study period but most patients had acceptable HbA_{1c} (< 7.0%), LDL-cholesterol (< 2.5 mmol/L) and ACR (< 3.0 mg/mmol) at entry. The ESS decreased significantly over the study period, specifically between entry and 1 month (Δ ESS = -4.8 (-6.5 to -3.1, $P < 0.001$)). Amongst the 27 males, the median number of symptoms of sexual dysfunction decreased significantly over the study period, from 6.5 to 5.5 ($P = 0.008$).

Conclusion: A 3-month trial of CPAP in community-based type 2 patients improved blood pressure, pulse rate, daytime sleepiness, and, in men, sexual function.

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